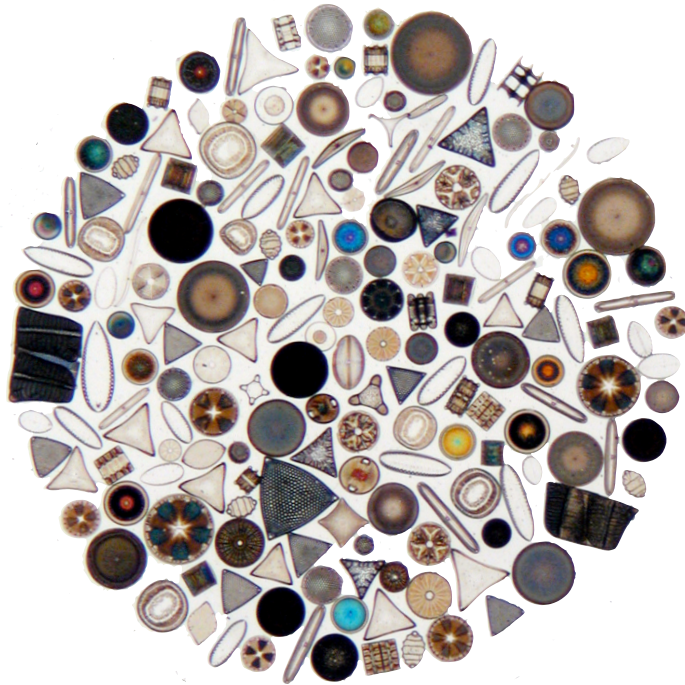


**Changes in nutrient stoichiometry:
phytoplankton & organic matter dynamics in
coastal upwelling systems**



Dissertation

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Summary

Coastal upwelling systems associated to the eastern continental margins of the Atlantic and Pacific Oceans are among the most productive realms of the marine ecosystems. Although they only occupy a small area, they play a globally important role in the cycling of nitrogen (N), phosphorus (P) and other biologically relevant elements. In subsurface waters of upwelling systems, oxygen minimum zones (OMZs) persist as a result of biological degradation and sluggish ventilation. Reduced oxygen concentrations influence redox sensitive nutrient inventories by promoting N loss processes and P release from the sediment. Hence, water masses upwelled to the surface feature low N:P ratios that deviate from canonical Redfield proportions of 16:1. Due to the excess P over N, upwelling systems are thought to favor the growth of dinitrogen (N₂) fixing organism (diazotrophs) that could potentially restore inorganic nutrient ratios back to Redfield proportions and replenish the N deficit in those waters. Contrary to this assumption, the presence of non-diazotrophic phytoplankton utilizing nutrients in lower than Redfield proportions has been suggested to eliminate the niche for diazotrophs. Thus, the dominance of either Redfield or non-Redfield primary production is thought to determine the amount of N fixed in upwelling systems.

In light of expanding OMZs and the predicted modification of nutrient inventories, this doctoral dissertation aimed to investigate the impact of changing N:P supply ratios on phytoplankton and organic matter composition. Moreover, the potential of primary producers to modify nutrient supply anomalies and their role in coupling or decoupling sources and sinks of fixed N was assessed. To accomplish this, nutrient manipulation experiments and a field study were conducted in the eastern tropical North Atlantic (ETNA) and eastern tropical South Pacific (ETSP).

To better understand the impact of changing N:P ratios on primary production and on N₂ fixation in the ETNA surface ocean, mesocosm experiments with natural plankton communities were carried out in the first study. Nutrient drawdown, bloom formation, biomass build-up and diazotrophic feedback mechanisms in response to variable N:P ratios were investigated. The obtained results indicate that N availability was the key factor determining primary production. Moreover, phytoplankton elemental composition depended on the organisms' growth phase rather than on initial nutrient supply ratios. A channeling of excess P into the dissolved organic phosphorus (DOP) pool was

observed in all experiments. Two findings strongly challenged the classical Redfield-based view on N_2 fixation: (i) the availability of inorganic N compounds did not negatively affect N_2 fixation and (ii) under P limitation, DOP seemed to provide an additional P source for diazotrophs.

In the second study, phytoplankton species distribution and organic matter composition was investigated during in situ measurements in the ETSP. Results from this study demonstrated that low inorganic N:P ratios in the water column were not mirrored in particulate organic matter ratios, which was contrary to previous studies in this region. Drawdown of excess P and accumulation of DOP was indicative of the same mechanism of P channeling into the dissolved organic pool that was observed in the mesocosm experiments. The presence of previously undetected diazotrophic pigments in the highly productive shelf region further pointed towards the depletion of excess P or DOP through N_2 fixation.

Motivated by the outcome of the first two studies, bioassay experiments were conducted in the third study to test the availability of selected organic and inorganic P sources to diazotrophs. The obtained results demonstrated that diazotrophs were able to sustain and increase N_2 fixation rates on all tested P compounds, highlighting their competitive advantage under conditions of P depletion and DOP availability.

Results from this doctoral dissertation indicate that excess P is consumed and partially converted to DOP in upwelling regions. Transformation of excess P to DOP and its consumption by diazotrophs supports the assumption of a local replenishment of the N deficit via N_2 fixation.

Zusammenfassung

Die küstennahen Auftriebsgebiete der östlichen Kontinentalränder von Atlantik und Pazifik gehören zu den produktivsten Ökosystemen der Ozeane. Trotz ihrer geringen Ausdehnung sind sie von globaler Bedeutung für den Stickstoff- (N) und Phosphorkreislauf (P) sowie für den Umsatz weiterer biologisch relevanter Elemente. Als Resultat biologischer Abbauprozesse und einer trägen Ventilation befinden sich in den mittleren Wasserschichten dieser Auftriebsgebiete Sauerstoffminimumzonen (SMZ). Niedrige Sauerstoffkonzentrationen beeinflussen das redoxsensitive Nährstoffinventar und begünstigen Stickstoffverlustprozesse und die Freisetzung von P aus dem Sediment. Entsprechend findet sich in den zur Oberfläche auftreibenden Wassermassen ein niedriges N:P Verhältnis, das vom Redfield-Verhältnis von 16:1 abweicht. Es wird angenommen, dass der P-Überschuss in Auftriebsgebieten das Wachstum von Organismen fördert, die molekularen Stickstoff (N_2) fixieren können. Diese Diazotrophen sind möglicherweise in der Lage das N Defizit in solchen Gebieten auszugleichen und so das Verhältnis anorganischer Nährstoffe wieder an das Redfield Verhältnis anzunähern. Dieser Vermutung steht die Theorie gegenüber, dass nicht-stickstofffixierendes Phytoplankton, welches Nährstoffverhältnisse kleiner Redfield nutzt, die ökologische Nische für Diazotrophe eliminiert. Daher wird davon ausgegangen, dass das Ausmaß der N_2 -Fixierung von der vorherrschenden Art der Primärproduktion (im oder unterhalb des Redfield-Verhältnisses) abhängig ist.

Angesichts der zunehmenden Ausdehnung von SMZ und der prognostizierten Änderung des Nährstoffinventars, war das Ziel der vorliegenden Dissertation den Einfluss von veränderten N:P Verhältnissen auf Phytoplankton und die Zusammensetzung organischer Materie zu bestimmen. Darüber hinaus wurde untersucht, ob Primärproduzenten das Potential haben Nährstoffanomalien zu modifizieren und welche Rolle sie in der Kopplung und Entkopplung von N-Quellen und -Senken spielen. Hierzu wurden Nährstoffmanipulationsexperimente und eine Feldstudie im tropischen Nordostatlantik und tropischen Südostpazifik durchgeführt. Um die Auswirkungen sich verändernder N:P Verhältnisse auf die Primärproduktion und die N_2 Fixierung im Oberflächenwasser des Nordost-Atlantiks zu erfassen, wurden im Rahmen der ersten Studie Mesokosmosexperimente mit natürlichen Planktongemeinschaften durchgeführt. Im Speziellen wurde der Aufbau der

Biomasse und die Reaktionsmechanismen von Diazotrophen auf sich ändernde N:P Verhältnisse untersucht. Die Ergebnisse dieser Studie deuten darauf hin, dass die Verfügbarkeit von N das Ausmaß der Primärproduktion bestimmt. Zudem war die elementare Zusammensetzung des Phytoplankton nicht vom initialen Nährstoffverhältnis, sondern vielmehr von der Wachstumsphase der Organismen abhängig. Ein Transfer von überschüssigem P in gelöstes organisches Phosphat (DOP) ließ sich in allen Experimenten beobachten. Zwei Studienergebnisse stellen die klassische Sichtweise einer Redfield-basierten N_2 -Fixierung in Frage: (i) die Verfügbarkeit von fixiertem N hatte keinen negativen Einfluss auf die N_2 -Fixierung und (ii) unter P-Limitierung schien DOP eine zusätzliche P-Quelle für Diazotrophe zu bieten.

In der zweiten Studie wurden mittels in situ Messungen die Verteilung von Phytoplanktonarten und die Zusammensetzung organischer Materie im südöstlichen Pazifik untersucht. Im Gegensatz zu bisherigen in dieser Region durchgeführten Studien ließ sich hier beobachten, dass niedrige N:P Verhältnisse in der Wassersäule sich nicht in der Zusammensetzung der partikulären organischen Materie spiegelten. Die Aufnahme überschüssigen P und die Akkumulation von DOP deuteten auf denselben Mechanismus des P-Transfers in den Pool gelösten organischen Materials hin, der auch schon in den vorangegangenen Mesokosmosexperimenten beobachtet werden konnte. Der Nachweis bis dato unentdeckter Pigmente von Diazotrophen in den hochproduktiven Schelfgebieten des Südost-Pazifiks deutet darauf hin, dass überschüssiges P oder DOP durch N_2 -Fixierung verbraucht wurde.

Motiviert von den Ergebnissen der ersten beiden Arbeiten, fanden im Zuge der dritten Studie Bioassay Experimente statt. Hier sollte die Verfügbarkeit verschiedener organischer und anorganischer P-Quellen für Diazotrophe getestet werden. Es zeigte sich, dass N_2 -Fixierung mit allen P-Quellen aufrechterhalten oder gesteigert werden konnte. Diese Beobachtung hebt den Konkurrenzvorteil von Diazotrophen bei reduzierter P-Konzentration und gesteigerter DOP-Verfügbarkeit hervor.

Die Ergebnisse dieser Dissertation deuten darauf hin, dass überschüssiges P in Auftriebsgebieten konsumiert und partiell zu DOP konvertiert wird. Die Transformation überschüssigen P zu DOP und sein Verbrauch durch Diazotrophe bekräftigt die Annahme einer lokalen Wiederaufstockung des N-Defizits durch N_2 -Fixierung.

General Introduction

Eastern boundary upwelling systems

At the eastern continental margins of the tropical and subtropical Atlantic and Pacific Ocean, cold, nutrient-rich water masses from depth are transported into the sunlit surface zone. The physical mechanism of coastal upwelling is well understood (e.g. Barber and Smith, 1981; Smith, 1995): alongshore winds are driven by large-scale atmospheric pressure gradients between continental low pressure cells and high pressure systems over the eastern subtropical ocean basins. These upwelling favorable winds drive currents, which flow equator-wards along the eastern continental boundaries. Due to the Coriolis force stemming from the Earth's rotation, an 'Ekman flow' (Ekman, 1905) is produced, which transports surface water away from the coast. To compensate for this imbalance, water masses from deeper layers are upwelled to the surface.

The four eastern boundary upwelling systems (EBUS) are the Canary- and Benguela upwelling systems in the Atlantic and their Pacific counterparts the California- and Humboldt upwelling systems. Although both spatial extent and upwelling intensity in these systems vary seasonally (Narayan et al., 2010), permanent upwelling zones remain year-round (Chavez and Messié, 2009), which are characterized by low surface temperatures and high concentrations of inorganic nutrients such as silicate, nitrate, phosphate and iron. The high nutrient supply supports 'new' primary production, as opposed to 'regenerated' production based on recycled nutrients (Dugdale and Goering 1967), and greatly enhances phytoplankton biomass (Pennington et al., 2006; Ryther, 1969), mainly consisting of large and fast growing diatoms (Bruland et al., 2005). The high abundance of primary producers forms the basis for a short, productive food chain with large zooplankton standing stocks (Chavez and Messié, 2009) and extensive populations of small pelagic fish like sardines and anchovies (Rykaczewski and Checkley, 2008). Although driven by large-scale wind and circulation patterns, upwelling is a mesoscale process confined to a narrow coastal band. EBUS only cover a small part of the ocean (<1%), but support around 5% of the global marine primary production (Carr, 2002) and ~25% of the world's fishery (Chavez and Messié, 2009; Pauly and Christensen, 1995). Thus, EBUS are among the most productive ecosystems in the world's oceans (Lachkar and Gruber, 2012) and are of great economical importance.

Oxygen minimum zones

A distinct feature of eastern boundary upwelling systems is the prevalence of permanently low dissolved oxygen (O_2) concentrations in the subsurface ocean between 100-900 m water depth (Karstensen et al., 2008; Fig.1).

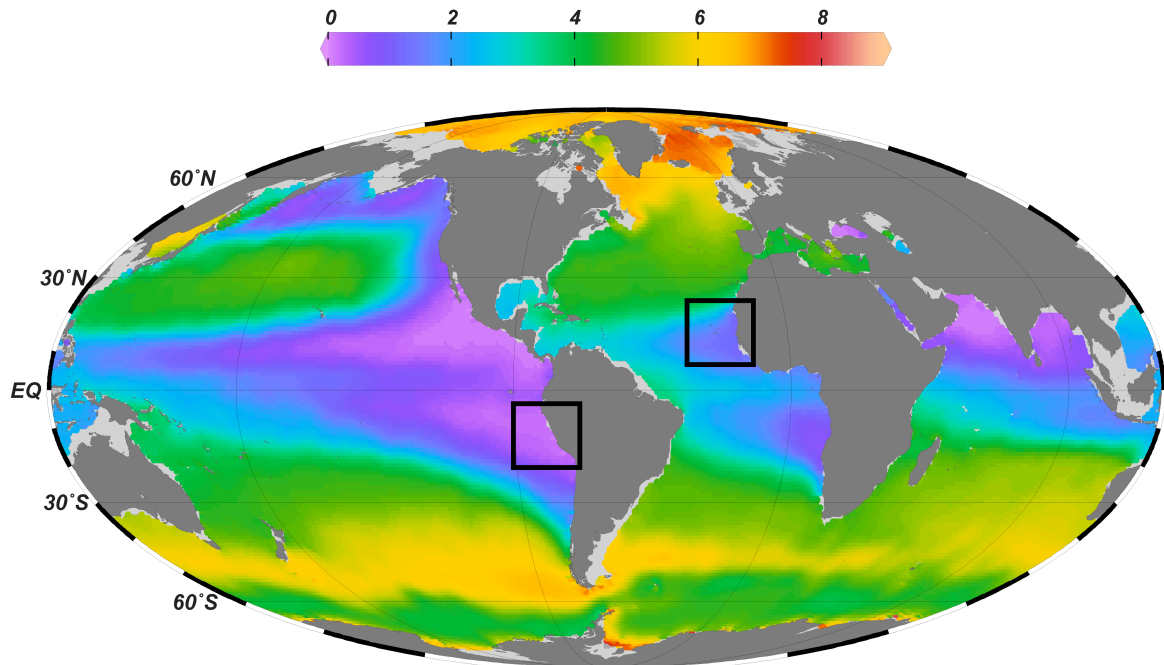


Figure 1: Global dissolved oxygen concentrations (mL L^{-1}) at 400 m water depth (annual mean). Black squares indicate the two areas investigated in this thesis: the eastern tropical South Pacific and eastern tropical North Atlantic. Data from World Ocean Atlas 2013 (NOAA) were plotted using Ocean Data View, <http://odv.awi.de>, Schlitzer, R., 2015.

The formation and maintenance of such oxygen minimum zones (OMZs) is a consequence of both physical and biogeochemical processes (Paulmier and Ruiz-Pino, 2009). Biogeochemical controls on O_2 concentrations in OMZs are mainly exerted through O_2 consumption via respiration and other chemical reactions depleting oxygen. High biological production in upwelling systems generates large amounts of organic material in surface waters, which subsequently sink into the ocean interior as detrital material (Muller-Karger et al., 2005). Here, microorganisms consume and degrade organic matter using O_2 as an electron acceptor. Oxygen is further depleted by oxidation of reduced molecules, such as sulfide and methane (Zhang et al., 2010). From the physical perspective, oxygen concentrations are low because subsurface water masses are poorly ventilated in the tropical and subtropical ocean. While oxygen in the surface is exchanged with the atmosphere and O_2 concentrations are maintained near saturation, water column stratification impairs vertical mixing and ventilation of the deeper water layers below the surface. Upwelled

source waters from the deep ocean are usually oxygen-poor as they have not been in contact with the atmosphere for a long time (centuries to millennia). Thus, O₂ concentrations are further reduced in mid-waters.

The terminology and thresholds defining oxygen deficiencies differ widely among studies (Riedel et al., 2016) and depend on whether the terms are used to describe sensitivities of marine taxa, microbial processes or biogeochemical cycling. Hypoxia is typically defined by a threshold of $\sim 60 \mu\text{mol kg}^{-1}$ (Gray et al., 2002). Below these concentrations many higher animal taxa are not able to survive (Vaquer-Sunyer and Duarte, 2008). O₂ values below $\sim 5 \mu\text{mol kg}^{-1}$ are termed suboxic and changes in biogeochemical cycling occur because O₂ is replaced by alternative electron acceptors (Ward et al., 2009). The term anoxic is used when O₂ levels drop to zero. Under these conditions anaerobic microbes, which convert sulfate to sulfide, tend to dominate the marine ecosystem (Keeling et al., 2010).

Oxygen concentrations in the eastern tropical Pacific OMZ are lowest, with mean values of $\sim 50 \mu\text{mol kg}^{-1}$ at 400 m water depth (Karstensen et al., 2008). Suboxic conditions are found at depths between 150–300 m and anoxia is often prevalent close to the shelf (Keeling et al., 2010). Compared to other OMZs, the Pacific Ocean also contains the largest volume of oxygen deficient water. In the North and South Pacific, 40% and 13% of the absolute volume is occupied by water masses with O₂ values below $90 \mu\text{mol kg}^{-1}$, respectively (Karstensen et al., 2008). Due to stronger ventilation, the Atlantic OMZ is not as oxygen-depleted or geographically expansive as its Pacific counterpart. In the North and South Atlantic, mean values of $\sim 100 \mu\text{mol kg}^{-1}$ and $\sim 50 \mu\text{mol kg}^{-1}$ can be found at 400 m depth, respectively (Karstensen et al., 2008) and no significant sub- or anoxic conditions occur. Water masses with O₂ values of $90 \mu\text{mol kg}^{-1}$ occupy approximately 5% and 7% of the North and South Atlantic, respectively.

As mentioned previously, O₂ levels in the sub- and anoxic range alter key pathways of biogeochemical processes and microbial activities (Doney and Karnauskas, 2014; Keeling et al., 2010; Paulmier and Ruiz-Pino, 2009), which in turn affect the composition and productivity of the ecological community. In particular nitrogen, iron and phosphorus cycling is influenced by low oxygen concentrations, thus the connection between O₂ and these nutrient cycles will be discussed in more detail hereafter.

The effect of low O₂ concentrations on nutrient cycling

Oxygen plays a critical role in the marine nitrogen (N) cycle, which includes various chemical species that exhibit a different degree of oxidation: ammonium (NH₄⁺), nitrite (NO₂⁻), nitrate (NO₃⁻), nitrous oxide (N₂O) and dinitrogen (N₂).

Under oxic conditions, NH₄⁺ is oxidized to NO₃⁻ in a process called nitrification. When O₂ concentrations fall below 5 μmol kg⁻¹, microbes – predominantly facultative anaerobic prokaryotes (Kuypers et al., 2005) – use NO₃⁻ instead of O₂ to oxidize organic matter (Codispoti et al., 2001). During this process, termed denitrification, nitrate is reduced to gaseous N₂O or N₂ via several intermediates: NO₃⁻ → NO₂⁻ → NO → N₂O → N₂. Thereby, bioavailable N (i.e. NO₃⁻, NO₂⁻, NH₄⁺) is lost from the oceans nutrient inventory, while organic matter is consumed and inorganic nutrients such as NH₄⁺ and phosphate (PO₄³⁻) are regenerated in order to sustain primary production (Ward et al., 2009). Another biochemical pathway removing fixed N from the environment under suboxic conditions was discovered in the beginning of the 21st century and is termed ‘anammox’: anaerobic ammonium oxidation (Dalsgaard et al., 2003; Kuypers et al., 2005). Anammox is performed by autotrophic bacteria that respire NH₄⁺ with NO₂⁻ to N₂. It is still under debate whether denitrification or anammox is primarily responsible for removing fixed nitrogen from the water column in OMZs. While denitrification is thought to be the major N loss process in the Arabian Sea OMZ (Bulow et al., 2010; Ward et al., 2009), anammox is presumed to dominate in the OMZ associated to the Benguela upwelling (Kuypers et al., 2005) and the Humboldt upwelling systems (Hamersley et al., 2007; Lam et al., 2009; Thamdrup et al., 2006; Ward et al., 2009). However, high denitrification rates were recently detected in subsurface waters off Chile and Peru, where maximum rates were up to an order of magnitude higher than those of anammox (Dalsgaard et al., 2012). In contrast to the Pacific, O₂ concentrations within the North Atlantic OMZ are too high to support denitrification or anammox, thus no water column N loss processes are thought to occur in this area (Karstensen et al., 2008; Ryabenko et al., 2012). Recent studies in mesoscale eddies in the eastern tropical North Atlantic (ETNA) are challenging this view, as extremely low O₂ concentrations in sub- and anoxic ranges were discovered in the cores of these eddies (Karstensen et al., 2015). A survey of the microbial community within one of these eddies indeed yielded the first indication of water column N loss in the ETNA (Löscher et al., 2015). Moreover, in the benthic environment off

Northwest Africa, ammonium-oxidizing bacteria have been detected (Jaeschke et al., 2010). These findings suggest that localized N loss processes might occur in the ETNA, but direct rate measurements are still missing.

Besides their critical role in the nitrogen cycle, OMZs also modulate the oceanic phosphorus (P) and iron (Fe) inventories, particularly where oxygen deficiency interacts with the sediment. In benthic environments, P is mainly bound to organic matter and fish debris. Moreover, P and Fe dynamics in the sediment are closely connected as a large P fraction is associated with iron oxyhydroxides (Hensen et al., 2006). Under reducing conditions, P is released from the sediment during the degradation of organic material and during the reduction of iron oxyhydroxides (Ingall and Jahnke, 1994; Noffke et al., 2012). The latter process also liberates dissolved ferrous iron (Fe^{2+}), which are then dispersed into the sediment-water interface (Scholz et al., 2014).

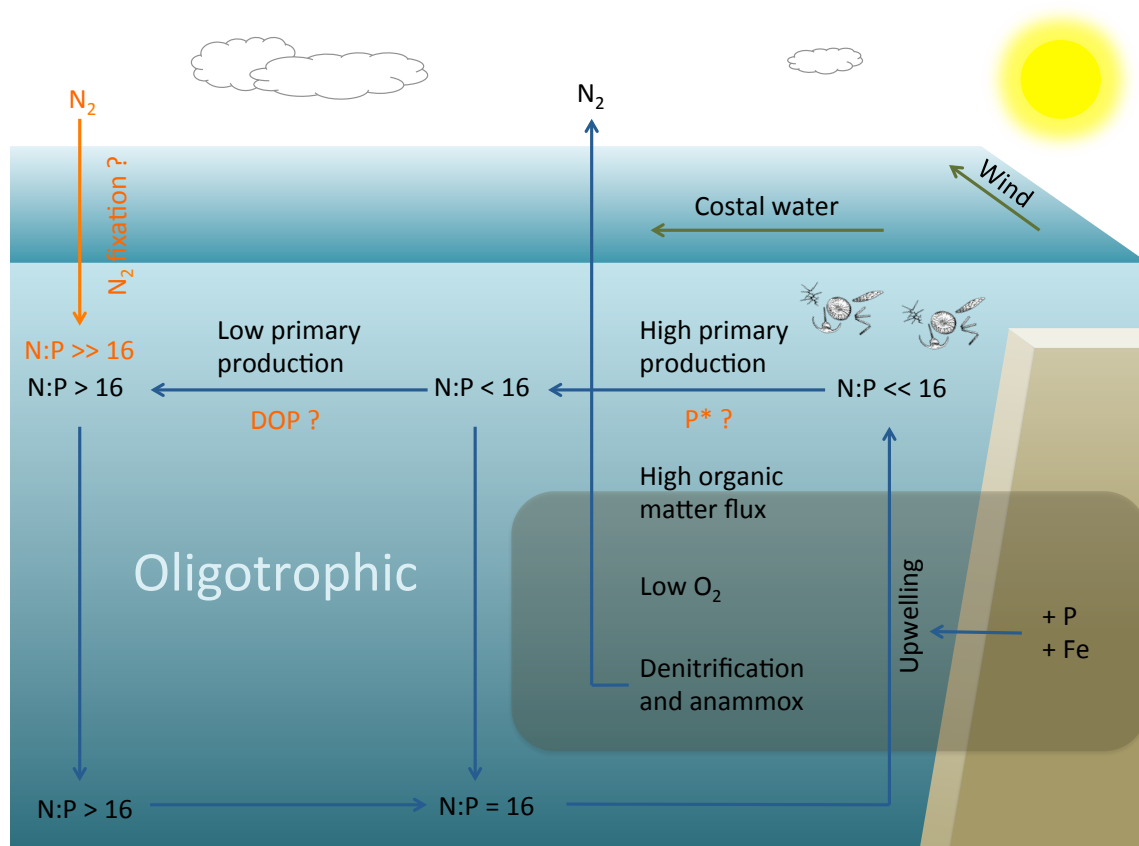


Figure 2: Schematic view of key nutrient fluxes within OMZs. Close to the shelf, primary production is high and a large amount of organic matter sinks to the ocean interior, where it is remineralized while O_2 is consumed. In these suboxic waters, anammox and denitrification convert NH_4^+ , NO_2^- and NO_3^- to N_2 , resulting in the loss of bioavailable N from the system. Additionally, Fe and P are released from the sediment under suboxic conditions, thus water masses upwelling to the surface are depleted in N relative to P. Further downstream, nutrient supply is diminished and primary production is low. It is still unknown through which process P^* is reduced as water masses are transported offshore and whether N_2 fixation replenishes the N deficit in upwelling regions.

In summary, OMZs are sinks for nitrogen, but provide a source of phosphorus (c.f. Fig. 2 for an overview of key nutrient fluxes in the realm of OMZs). As a result, an excess of P (P^*) over N is present in the water column, which is defined as:

$$P^* = PO_4^{3-} - NO_3^- / 16 \quad (1)$$

after Deutsch et. al (2007).

Because of severe oxygen depletion and on-going N loss processes in the Pacific OMZ, P^* values in this area are high (Fig. 3). In contrast, P^* values are close to zero in the Atlantic OMZ, where the moderate oxygen deficiency prevents large-scale N loss.

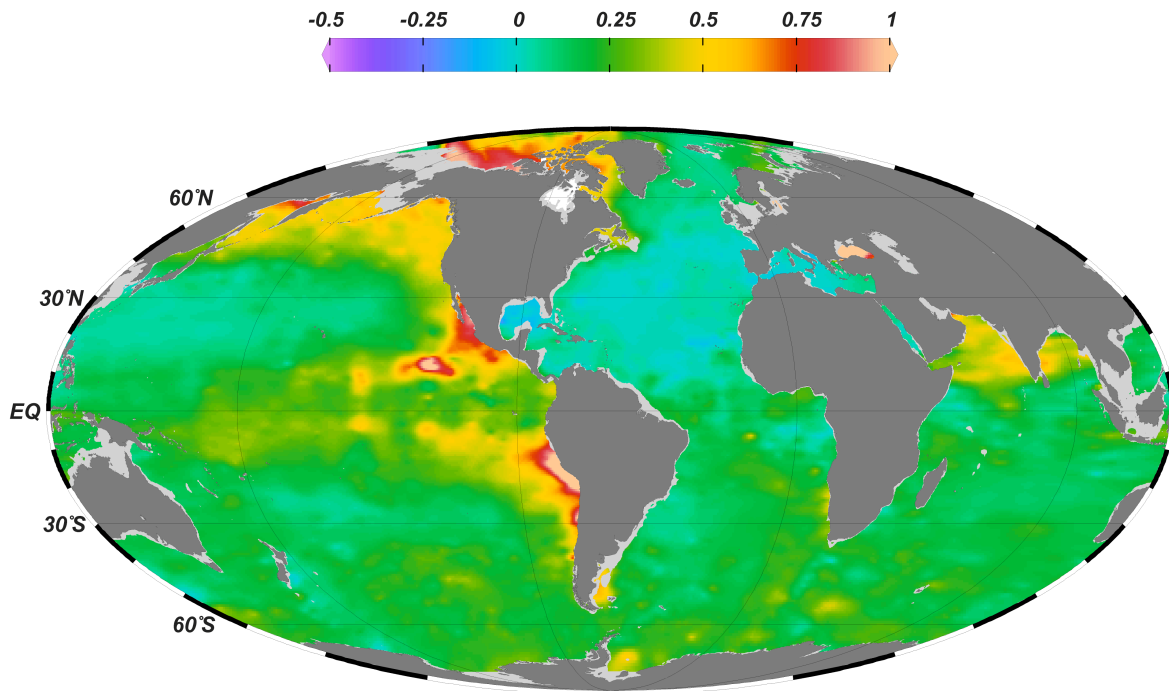
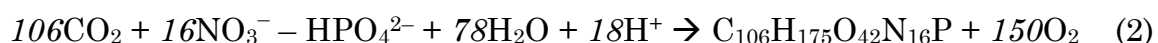


Figure 3: Mean annual distribution of P^* ($\mu\text{mol L}^{-1}$) at 100 m water depth, calculated as $P^* = PO_4^{3-} - NO_3^- / 16$ after Deutsch et al. (2007). Positive values indicate an excess of phosphate over nitrate relative to canonical Redfield proportions. Data from World Ocean Atlas 2013 (NOAA) were plotted using Ocean Data View, <http://odv.awi.de>, Schlitzer, R., 2015.

Phytoplankton production and nutrient stoichiometry

Phytoplankton are suspended photosynthetic microorganisms living in the euphotic (i.e. sunlit) layer of oceans and freshwater ecosystems. They produce organic matter during photosynthesis, a process where inorganic nutrients and carbon dioxide are transformed into organic biomass and oxygen using light energy and water as reducing agent (c.f. Eq. 2). As primary producers they are the main source of energy for heterotrophic consumers in the pelagic ecosystem and form the basis of the aquatic food web. Thus, they have a critical ecological function connecting all marine organisms to the abiotic ocean environment and contribute to the biogeochemical cycling of important chemical elements.

The most important inorganic nutrients required for phytoplankton growth are carbon (C), nitrogen and phosphorus. While C is a structural cell component, providing the fundament for carbohydrates and lipids, N is a key component of amino acids, which are the building blocks of all proteins. P is a major constituent of the cell membrane, of energy molecules such as adenosine triphosphate (ATP) and of DNA and RNA. The uptake of N and P by phytoplankton is closely linked to the fixation of carbon during photosynthesis. In 1958, Redfield observed that the average proportion of the three major elements C, N and P is remarkably similar in phytoplankton and in the deep ocean (Redfield, 1958). This led to the widespread view that these elements tend to be incorporated in a relatively fixed ratio by phytoplankton:



Correspondingly, Redfield concluded that the inorganic nutrient ratios in the oceans interior are controlled by the requirements of phytoplankton that ultimately sink and are remineralized at depth. Redfield's early observations have been confirmed several times (e.g. Anderson and Sarmiento, 1994; Goldman et al., 1979) and the Redfield Ratio of C:N:P of 106:16:1 remains a fundamental concept in marine ecology and biogeochemistry (Sterner and Elser, 2002).

However, it is known that under certain conditions inorganic nutrient stoichiometries (i.e. the proportion of the constituents N and P) shift away from the Redfield Ratio. These processes include dinitrogen (N₂) fixation, which adds fixed N in excess of P to the aquatic environment; as well as denitrification and anammox, which both remove fixed N from the water,

thereby leaving behind an excess of P over N. Based on Liebig's law of the minimum (Liebig, 1842), it has been suggested that the nutrient potentially controlling primary production can be predicted from nutrient concentrations in seawater (Kirkkala et al., 1998; Smith, 1984). Divergence from Redfield stoichiometry are then used as an indication of which nutrient is limiting (Ammerman et al., 2003). Others suggested that the abundance of certain phytoplankton species is determined by the relative proportion of nutrients in seawater and not by their absolute concentration (Tilman, 1982). Specific phytoplankton species are expected to dominate at their optimal resource ratio and will be replaced by others when the nutrient stoichiometry changes (Tilman, 1982). However, many authors argue that the Redfield Ratio is not the universal and constant stoichiometry of phytoplankton, but is only an average optimal ratio for the whole phytoplankton community (Deutsch and Weber, 2012; Falkowski, 2000; Klausmeier et al., 2008; Lagus, 2004).

Indeed, it is increasingly recognized that inorganic nutrient stoichiometries marking the point of nutrient limitation and phytoplankton cellular N:P ratios differ greatly within and between taxa, with growth conditions and with growth strategies (Table 1 provides an overview of studies investigating the connection between inorganic N:P ratios and phytoplankton elemental stoichiometries). On the one hand, species-specific differences in cellular stoichiometries seem to be determined by the microorganisms' phylogenetic background. Quigg et al. (2003) showed that C:N:P ratios vary substantially between phyla that belong to different superfamilies. For example, chlorophytes and prasinophytes belonging to the green superfamily exhibit mean N:P ratios of ~30:1. In contrast, diatoms and prymnesiophytes are members of the red superfamily and have lower N:P ratios of about 10:1. On the other hand, the relative contributions of cellular constituents such as phospholipids, ATP, DNA/RNA, chlorophyll and amino acids were shown to determine the overall N:P ratio of phytoplankton (Geider and La Roche, 2002; Gillooly et al., 2005; Sterner and Elser, 2002). As photoautotrophs invest the majority of N into proteins and the majority of P into ribosomal RNA, these two cellular components seem to be the major contributors to cellular N:P ratios in primary producers (Falkowski, 2000; Geider and La Roche, 2002; Klausmeier et al., 2004; Loladze and Elser, 2011). Another central paradigm of ecological stoichiometry is the growth rate hypothesis (Elser et al., 1996). It states that growing organisms need to increase their uptake of P to satisfy the elevated demand required for RNA synthesis. Based in this concept and

theoretical work by Klausmeier et al. (2004), Arrigo (2005) developed the notion of ‘survivalists’ and ‘bloomers’. In short, he suggests that fast growing phytoplankton (‘bloomers’) contain a high amount of growth machinery (i.e. rRNA) and thus exhibit low N:P ratios, while slow-growing phytoplankton have a proportionally high amount of resource acquisition machinery (i.e. chlorophyll and proteins), exhibiting a high N:P ratio. He concluded that certain environmental conditions select for the dominance of phytoplankton of either of these two groups, with N:P supply ratios determining the abundance of ‘bloomers’ and ‘survivalists’ (Arrigo, 2005).

In coastal upwelling zones, fast growing diatoms are known to dominate the phytoplankton community (Bruland et al., 2005; Chavez et al., 1996; Franz et al., 2012b). Since these organisms have been shown to exhibit low cellular N:P ratios (Quigg et al., 2003; Weber and Deutsch, 2010), they are thought to consume the excess of phosphate present in the water column via non-Redfield

Table 1: Overview of major studies investigating the connection between inorganic nutrient stoichiometries (N:P_i) and cellular stoichiometries of phytoplankton (N:P_{Phyt}).

Study	Study-type	N:P _i influences N:P _{Phyt}	N:P _{Phyt} independent from N:P _i	Major finding/theory	Specifics
Goldman 1979	Laboratory experiments	√		N:P _{Phyt} = 16:1 under maximum growth	Variable N:P _{Phyt} under nutrient limitation
Tilman 1982	Theoretical work	√		Resource ratio hypothesis	Species dominate at their optimal N:P _i
Redfield 1985	Theoretical work and review		√	Redfield Ratio	Phytoplankton requirements control N:P _i
Geider and La Roche 2002	Review	√		N:P _{Phyt} depends on growth condition	Nutrient replete: low N:P _{Phyt} , nutrient limited: high N:P _{Phyt}
Sterner and Elsner 2002	Review	√		Growth rate hypothesis	Growth rate and cellular phosphorus content correlate positively
Quigg 2003	Laboratory experiment		√	Phylogenetic dependence of N:P _{Phyt}	Green superfamily: high N:P _{Phyt} , red superfamily: low N:P _{Phyt}
Klausmeier 2004	Modeling	√		N:P _{Phyt} influenced by growth strategy	Nutrient replete: high N:P _{Phyt} , nutrient limited: low N:P _{Phyt}
Arrigo 2005	Review	√		Bloomers’ and ‘Survivalists’	Nutrient replete: high N:P _{Phyt} , nutrient limited: low N:P _{Phyt}
Hall 2005	Field and laboratory experiments		√	N:P _i is not reflected in N:P _{Phyt}	N:P _{Phyt} constant over wide N:P _i ranges
Weber and Deutsch 2010	Field data and modeling		√	N:P _i governed by phytoplankton biogeography	Species composition and distribution influences N:P _i
Loladze and Elser 2011	Modeling and review	√		Redfield Ratio rooted in protein-to-RNA ratio	Nutrient replete: 16:1 _{Phyt} , nutrient limited: variable N:P _{Phyt}
Hillebrand et al. 2013	Meta-analysis	√		N:P _{Phyt} more restricted under nutrient replete conditions	Nutrient replete: low N:P _{Phyt} , nutrient limited: variable N:P _{Phyt}

nutrient utilization (Mills and Arrigo, 2010). As the availability of phosphate controls the distribution of N_2 fixing organisms, this would have major implications for the N cycle since the relative abundance of fast- and slow-growing phytoplankton would control the amount of new nitrogen added to the ocean. This feedback will be discussed in more detail in the next section.

Regardless of whether phytoplankton cellular stoichiometry is ultimately controlled by growth rate, growth strategy or nutrient supply ratio, the influence of dissolved organic matter (DOM) on phytoplankton production and elemental composition has often been overlooked. DOM can be a major reservoir of N and P in the surface ocean, both in eutrophic (Davis et al., 2014) and oligotrophic realms (Reynolds et al., 2014; Torres-Valdés et al., 2009). Therefore, DOM potentially plays an important role in sustaining phytoplankton growth, especially in areas where primary production is limited by inorganic nutrients (Mather et al., 2008; Sañudo-Wilhelmy et al., 2001). Due to its chemical complexity, DOM is not yet fully characterized and remains largely under-sampled, but evidence suggests that the stoichiometry of DOM differs drastically from Redfield proportions in many areas of the ocean (Deutsch and Weber, 2012; Hopkinson and Vallino, 2005). Studies investigating the elemental composition of DOM suggest that dissolved organic phosphorus (DOP) is more reactive and is remineralized faster than dissolved organic nitrogen (DON). Thus, DON and DOP seem to be less coupled compared to the inorganic and particulate organic forms of N and P. Moreover, the spatial distribution of DOP is more variable than that of DON, both in the Pacific (Abell et al., 2000; Raimbault et al., 2008) and the Atlantic (Landolfi et al., 2016; Letscher et al., 2013; Torres-Valdés et al., 2009). The apparent differences in production and remineralization of DON and DOP are thought to strongly affect DOM stoichiometry. As primary producers and N_2 fixers seem to be able to utilize certain DOM compounds – especially DOP (Dyhrman et al., 2006; Mahaffey et al., 2014; Sohm and Capone, 2006) – dissolved organic matter concentration and lability might be a crucial factor influencing nutrient supply and uptake by phytoplankton in the surface ocean.

Nitrogen fixation and the diazotrophic niche in upwelling regions

The availability of fixed N is considered to limit oceanic primary productivity on a global scale (Gruber, 2004). The fixation of N_2 is the dominant source of bioavailable N to the marine environment (Benavides and Voss, 2015;

Falkowski et al., 1998) and the only natural process that counterbalances N loss by denitrification and anammox (Gruber and Sarmiento, 1997).

Biological nitrogen fixation occurs through the conversion of atmospheric N₂ into ammonium, which is performed by a variety of prokaryotes called diazotrophs (Capone et al., 2008). The reduction of N₂ is an energetically costly process: the nitrogen molecule consists of two N atoms that are bound through a triple covalent bond, making the molecule highly inert and non-reactive. The breaking of this bond is catalyzed by the nitrogenase enzyme, which consists of two proteins containing iron and molybdenum (Postgate, 1982). Since the biological N₂ fixation pathway is so energy-intensive (~16 moles of ATP are required to reduce one mole of N₂), diazotrophs grow slowly in comparison to other phytoplankton species and are considered to be outcompeted by other primary producers when nutrients are abundant (Bonnet et al., 2009; Monteiro et al., 2011). Thus, their classical niche is set in areas where low bioavailable N hampers the production of non-diazotrophs and their requirements for phosphorus and iron are met (Tyrrell, 1999). Phosphate and iron-rich upwelled waters are thought to provide such a niche for N₂ fixers (Deutsch et al., 2007). The premise is that nutrient assimilation by non-diazotrophs in the highly productive surface ocean is according to Redfield and residual phosphate is left behind (Deutsch et al., 2007; Tyrrell, 1999). This hypothesis is in direct contrast to the assumption of non-Redfield production (e.g. by diatoms) in upwelling regions (Mills and Arrigo 2010). As mentioned before, the availability of fixed nitrogen depends on the differences between loss processes and fixation (Gruber and Galloway, 2008) and on the distribution and magnitude of diazotrophic activity in the ocean (Benavides and Voss, 2015; Weber and Deutsch, 2014). If N loss processes were coupled both geographically and temporally to N₂ fixation through P* as suggested by (Deutsch et al., 2007), this feedback cycle would balance the marine N budget. If the abundance of diazotrophs is, however, controlled by non-diazotrophs consuming P*, N loss and gain processes would be spatially and temporally decoupled (Mills and Arrigo, 2010). This could lead to a perturbation of the N cycle steady-state, resulting in an unbalanced marine N budget, as has previously been suggested by others (Codispoti, 2007; Codispoti et al., 2001; Galloway et al., 2004).

EBUS and climate change

Fossil fuel emissions and increasing anthropogenic carbon dioxide (CO₂) concentrations in the atmosphere lead to ocean acidification, rising surface ocean temperatures and deoxygenation (Gruber, 2011). As these changes intensify, the oceanic environment will be fundamentally changed, with potentially serious consequences for marine ecosystems. Increasing stratification and decreased oxygen solubility due to ocean warming are affecting OMZs in particular, with an expansion and shoaling of low oxygen waters already being observed (Stramma et al., 2008; 2009; 2010). For the next century, modeling approaches have predicted a further decline of the global ocean oxygen inventory between 1 and 7% (Keeling et al. 2011). Reduced oxygen levels pose a threat to a variety of marine organisms in benthic and pelagic habitats that respire aerobically (Rabalais et al., 2010; Vaquer-Sunyer and Duarte, 2008). A reduction of habitable areas could cause shifts in species diversity and food web structure in these regions (Wishner et al., 2013).

Moreover, decreasing levels of dissolved oxygen could further increase denitrification and anammox processes, with substantial consequences for the marine nitrogen inventory (Lam and Kuypers, 2011). Not only do these processes contribute to marine N loss via the regeneration of N₂, denitrification also produces nitrous oxide (N₂O) (Arévalo-Martínez et al., 2015; Naqvi et al., 2010). N₂O is a strong greenhouse gas and ~300 times more potent than CO₂ (Ravishankara et al., 2009), thus an increased production could potentially amplify global warming (Gruber and Galloway, 2008).

With a possible facilitation of N loss processes in OMZs, the N deficit in these waters might increase further in the future. The oxygen depleted areas in the tropical North Atlantic could encounter strong changes, where a moderate O₂ deficiency has prevented extensive N loss processes to date (Karstensen et al., 2008). An enhancement of N loss processes in upwelled waters could result in a decline of total primary production, as phytoplankton were shown to be primarily limited by the availability of N in these areas (Codispoti et al., 2001; Franz et al., 2012a; Gruber, 2004). This decline could further be enhanced by higher stratification, reducing the effective provision of nutrients from upwelling systems (Gruber, 2011), with unknown consequences for the highly productive and economically important ecosystems of upwelling regions.

Motivation and thesis outline

Open questions and major objectives

Despite our increasing knowledge regarding the influence of variable nutrient stoichiometries on the production and composition of organic matter, there are still major uncertainties concerning the role of changing nutrient supply ratios in upwelling regions. Especially in light of climate change and the predicted increase in N loss processes within OMZs, it is crucial to assess the role of phytoplankton in modulating biogeochemical cycles of N and P. Specifically, uncertainties remain about the connection between different N turnover processes in OMZ influenced water masses. Although Deutsch (2007) suggested that N loss processes are spatially and temporally coupled to nitrogen fixation in upwelling regions, other authors suggested that P^* is utilized via non-Redfield production, thus removing the niche for diazotrophs (Mills and Arrigo, 2010). Field studies investigating the mechanisms of P^* removal in upwelling regions are scarce, but Franz et al. (2012b) found that non-diazotrophs reduced P^* via non-Redfield production in the Peruvian upwelling system. In a separate study these authors found that P^* was channeled through the particulate organic phosphorus into the DOP pool during non-Redfield nutrient utilization (Franz et al., 2012a). As diazotrophs are thought to be available to assimilate DOP (Dyhrman et al., 2006; Sohm and Capone, 2006), N_2 fixation might be stimulated by enhanced DOP supply under low nutrient N:P ratios (c.f. Fig. 2 for an overview of potential N and P fluxes in upwelling regions). Thus, more information on the availability and fate of DOP in upwelling regions is extremely valuable.

A major aim of this thesis was to address fundamental questions on how variable nutrient stoichiometries influence primary producers and the composition of particulate and dissolved organic matter pools. Another goal was to assess the mechanism of P^* removal in upwelling regions and to determine the role of DOP in P^* consumption and as a nutrient source for diazotrophs. These questions were tackled within two major upwelling regions that show some distinct biogeochemical differences: the eastern tropical North Atlantic and the eastern tropical South Pacific.

Chapter I presents results from two consecutive mesocosm experiments that investigated the effect of changing nutrient stoichiometries as a consequence of ocean deoxygenation on biomass production and diazotrophy in the ETNA. In mesocosms with low N:P supply ratios, the role of excess P on phytoplankton growth and particulate and dissolved organic matter production was investigated. Phosphorus dynamics and their influence on N₂ fixation were monitored. Since the availability of fixed N is suggested to inhibit N₂ fixation, high N:P supply ratios were applied to assess the effect of bioavailable N on N₂ fixation. To characterize the diazotrophic community, we conducted direct N₂ fixation rate measurements and determined *nifH* gene and transcript abundances.

Chapter II contains results from in situ measurements in the ETSP investigating the phytoplankton species distribution and organic matter composition in water masses featuring low inorganic N:P ratios. We examined spatial P* and DOP dynamics on transects off the Peruvian coast to ascertain the mechanisms responsible for P* removal and to investigate the fate of DOP in upwelling region. Furthermore, we conducted pigment composition analyses via high performance liquid chromatography (HPLC) to assess the distribution of diazotrophic and non-diazotrophic phytoplankton.

Chapter III reports on the results from nutrient incubation experiments in the ETNA, where we investigated the response of the natural phytoplankton community to amendments with different phosphorus compounds. At six stations, surface seawater was incubated with either dissolved inorganic phosphate (DIP) or one of two different DOP sources to assess whether DOP can be utilized by diazotrophs and stimulates N₂ fixation and whether bioavailability differs between DIP and DOP compounds.

First-author papers and declaration of contribution

Chapter I

Meyer, J., Löscher, C. R., Neulinger, S. C., Reichel, A. F., Loginova, A., Borchard, C., Schmitz, R. A., Hauss, H., Kiko, R. and Riebesell, U.: Changing nutrient stoichiometry affects phytoplankton production, DOP accumulation and dinitrogen fixation – a mesocosm experiment in the eastern tropical North Atlantic, *Biogeosciences*, 13(3), 781–794, doi:10.5194/bg-13-781-2016, 2016.

Idea and experimental design: Helena Hauss and Rainer Kiko with input from Judith Meyer, Carolin Löscher, Alexandra Loginova, Corinna Borchard, Ulf Riebesell and Ruth Schmitz-Streit

Data acquisition: Judith Meyer, Rainer Kiko, Anna Reichel, Alexandra Loginova, Corinna Borchard and Helena Hauss

Data analyses: Judith Meyer, Carolin Löscher and Anna Reichel

Manuscript preparation: Judith Meyer and Carolin Löscher in equal contribution with comments from all co-authors

Chapter II

Meyer J., Lavik, G. and Riebesell, U.: The effect of nutrient stoichiometry on organic matter dynamics, phytoplankton community composition and diazotrophy in the eastern tropical South Pacific. Under review in *Frontiers in Marine Science*.

Idea: Judith Meyer and Ulf Riebesell

Data acquisition: Judith Meyer and Gaute Lavik

Data analyses: Judith Meyer and Gaute Lavik

Manuscript preparation: Judith Meyer with comments from Gaute Lavik and Ulf Riebesell

Chapter III

Meyer, J., Singh, A. and Riebesell, U.: Dissolved phosphorus compounds enhance N₂ fixation rates in the eastern tropical North Atlantic. *To be submitted*.

Idea and experimental design: Judith Meyer and Ulf Riebesell

Data acquisition: Judith Meyer and Arvind Singh

Data analyses: Judith Meyer

Manuscript preparation: Judith Meyer with comments from Arvind Singh and Ulf Riebesell

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**Changing nutrient stoichiometry affects
phytoplankton production, DOP accumulation
and dinitrogen fixation –
a mesocosm experiment in the eastern tropical
North Atlantic**

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Abstract

Ocean deoxygenation due to climate change may alter redox-sensitive nutrient cycles in the marine environment. The productive eastern tropical North Atlantic (ETNA) upwelling region may be particularly affected when the relatively moderate oxygen minimum zone (OMZ) deoxygenates further and microbially driven nitrogen (N) loss processes are promoted. Consequently, water masses with a low nitrogen to phosphorus (N:P) ratio could reach the euphotic layer, possibly influencing primary production in those waters. Previous mesocosm studies in the oligotrophic Atlantic Ocean identified nitrate availability as a control of primary production, while a possible co-limitation of nitrate and phosphate could not be ruled out. To better understand the impact of changing N:P ratios on primary production and N₂ fixation in the ETNA surface ocean, we conducted land-based mesocosm experiments with natural plankton communities and applied a broad range of N:P ratios (2.67–48). Silicic acid was supplied at 15 $\mu\text{mol L}^{-1}$ in all mesocosms. We monitored nutrient drawdown, biomass accumulation and nitrogen fixation in response to variable nutrient stoichiometry. Our results confirmed nitrate to be the key factor determining primary production. We found that excess phosphate was channeled through particulate organic matter (POP) into the dissolved organic matter (DOP) pool. In mesocosms with low inorganic phosphate availability, DOP was utilized while N₂ fixation increased, suggesting a link between those two processes. Interestingly this observation was most pronounced in mesocosms where nitrate was still available, indicating that bioavailable N does not necessarily suppress N₂ fixation. We observed a shift from a mixed cyanobacteria/proteobacteria dominated active diazotrophic community towards a diatom-diazotrophic association of the *Richelia-Rhizosolenia* symbiosis. We hypothesize that a potential change in nutrient stoichiometry in the ETNA might lead to a general shift within the diazotrophic community, potentially influencing primary productivity and carbon export.

Introduction

Eastern boundary upwelling systems are characterized by cold, nutrient-rich water masses that are transported from intermediate water layers towards the surface. The resulting extensive primary production forms the basis for high biomass development and a productive food web (Pennington et al., 2006). At the same time, biological degradation at depth and weak interior ventilation cause permanently low oxygen concentrations in intermediate water masses (100–900 m, Karstensen et al., 2008). These low oxygen conditions support denitrification and anammox that remove bioavailable nitrogen (N) from the water column (e.g. Codispoti et al., 2001; Kalvelage et al., 2011; Lam et al., 2009). Oxygen minimum zones (OMZs) also influence the availability of inorganic phosphate (P), silicon (Si) and trace elements such as iron (Fe), which are released at the sediment-water interface under oxygen-deficient conditions (Hensen et al., 2006; Ingall and Jahnke, 1994). Subsequently, the elemental stoichiometry of inorganic nutrients (N:P) in upwelled water masses is below the Redfield ratio of 16:1 (Redfield, 1958), which manifests itself as an excess of P (P^*) relative to N ($P^* = \text{PO}_4^{3-} - \text{NO}_3^-/16$, after Deutsch et al. 2007). In the eastern tropical North Atlantic (ETNA) nutrient concentrations and stoichiometry within the euphotic layer cover a wide range. Water masses in coastal regions feature low N:P ratios mainly as a result of benthic N loss along with P leaching from the sediment (Jaeschke et al., 2010; Schafstall et al., 2010; Trimmer and Nicholls, 2009), suggesting an N limitation of primary production in OMZ-influenced surface waters (Deutsch et al. 2007). In the transition zone between coastal upwelling and open ocean, N:P ratios approach Redfield proportions (Moore et al., 2008). Nevertheless, the nitracline tends to be deeper than the phosphocline in the ETNA (Hausse et al., 2013; Sandel et al., 2015), which also points towards a deficiency of N over P in the euphotic zone. In the Central and West Atlantic, N:P ratios beyond 30:1 can be reached (Fanning, 1992; Moore et al., 2008), suggesting a severe P limitation of primary producers (Ammerman et al., 2003; Mills et al., 2004). Additional input of atmospheric anthropogenic nitrogen into the open ocean could further increase this P deficit in the future (Duce et al., 2008). Oxygen concentrations within the oxygen minimum in the ETNA are usually above $40 \mu\text{mol kg}^{-1}$ and thus considered too high to support N loss processes in the water column (Karstensen et al., 2008; Löscher et al., 2012; Ryabenko et al., 2012). However, recent observations of very low oxygen levels just below the mixed layer

associated to anticyclonic medowater eddies suggest a potential for localized denitrification – with an accompanied decrease in N:P ratios – in the open ocean of the ETNA (Karstensen et al., 2015).

Discrepancies from the canonical N:P ratio are known to influence productivity and composition of primary producers (Grover, 1997). Since the average elemental composition of N and P in seawater as well as in phytoplankton is 16:1, a deviation of dissolved inorganic nutrients from this ratio could indicate which nutrient can potentially become limiting before the other (Lagus, 2004; Moore et al., 2013). Transferring this concept to upwelling regions with inorganic N:P ratios below Redfield, one would expect that the limiting nutrient for phytoplankton growth in those areas is N. It has been shown, however, that certain functional ecotypes of phytoplankton differ in their required nutrient ratio, as specific cellular entities (e.g. chlorophyll, proteins or rRNA) of primary producers have a unique stoichiometric composition deviating from the classical Redfield stoichiometry (Arrigo, 2005; Geider and La Roche, 2002; Quigg et al., 2003). Thus, surface waters adjacent to OMZs potentially provide a niche for certain types of primary producers, whose growth strategy and metabolic requirements are favored by low ratios of N:P. Arrigo (2005) refers to them as ‘bloomers’ and characterizes them as organisms adapted to exponential growth, which contain high amounts of ribosomes and P rich rRNA. Those organisms build their biomass in non-Redfield proportions and exhibit low cellular N:P ratios. The deficit in inorganic N of water masses adjacent to OMZs would thus be reduced by this non-Redfield production and N:P ratios further offshore would approach Redfield conditions.

Another concept of phytoplankton growth in N-deficient waters is that inorganic nutrients are taken up in Redfield proportion by primary producers, which leaves the surface water masses enriched in P. Excess phosphate presence has been hypothesized to favor N₂ fixation (Deutsch et al., 2007). The conversion of readily available dissolved N₂ into bioavailable forms of fixed N by diazotrophs could replenish the N-deficit in surface waters adjacent to OMZs.

Previous bioassay studies that were conducted to identify controlling factors for primary production in the eastern Atlantic using inorganic N, P and dissolved Fe addition, determined N as the key limiting nutrient (e.g. Graziano et al., 1996; Mills et al., 2004; Moore et al., 2008). These findings are in accordance with an on-board mesocosm study from the same area, where phytoplankton growth depended on the initial supply of N rather than on the

N:P ratio and where a combined addition of N and P did not further increase biomass production compared to the addition of N sources alone (Franz et al., 2012). Additionally, the authors deduced that at low N:P ratios excess P was assimilated by non-diazotrophic phytoplankton and was channeled into dissolved organic phosphorus (DOP). As DOP might serve as an additional source of P for bacteria and phytoplankton (Mahaffey et al., 2014 and references therein) and is preferentially taken up by the filamentous diazotrophic cyanobacterium *Trichodesmium* (Dyhrman et al., 2006; Sohm and Capone, 2006), it has been proposed that N₂ fixation might be stimulated by an enhanced DOP supply under low N:P ratios (Franz et al., 2012).

Until recently, oceanic N₂ fixation was mainly attributed to phototrophic cyanobacteria, such as *Trichodesmium* or *Crocospaera*, which are restricted to nutrient depleted surface to subsurface waters due to their light demand (Capone et al., 1997; Zehr and Turner, 2001). However, several groups of non-cyanobacterial diazotrophs and cyanobacterial symbionts have been detected in various oceanic regions, thus demonstrating the ubiquity and high diversity of diazotrophs (Farnelid et al., 2011; Foster et al., 2009; Loescher et al., 2014). Despite the growing awareness of diazotrophic diversity and distribution, the environmental conditions controlling diazotrophy are still not well understood. However temperature, Fe and P availability and dissolved oxygen concentrations are regarded as key factors for diazotrophic distribution and partly for active N₂ fixation (e.g. Sohm et al., 2011). The presence of high amounts of fixed N is thought to inhibit N₂ fixation (Weber and Deutsch, 2014), since diazotrophs are either outcompeted by fast growing phytoplankton species such as diatoms (Bonnet et al., 2009; Monteiro et al., 2011), or they themselves take up bioavailable forms of N rather than use the energy consuming process of N₂ fixation (Dekaezemacker and Bonnet, 2011; Mulholland and Capone, 2001; Mulholland et al., 2001).

In the ETNA, upwelling of N depleted waters along with high Fe input via Saharan dust deposition (Gao et al., 2001) sets a classical niche for N₂ fixation, while high N:P ratios beyond the upwelling region of the ETNA point towards P limitation of diazotrophs (Ammerman et al., 2003; Mills et al., 2004). Nevertheless, a diverse community of cyanobacterial diazotrophs such as *Trichodesmium* (Capone et al., 1997; Tyrrell et al., 2003), a variety of unicellular cyanobacterial diazotrophs (Groups A, B, C, diatom-symbionts) (Falcon et al., 2002; Langlois et al., 2005) as well as non-cyanobacterial diazotrophs such as different clades of proteobacteria are abundant and widely

distributed (e.g. Langlois et al., 2005; 2008). Those diazotrophs have previously been demonstrated to actively fix N_2 in the ETNA (Foster et al., 2009; Langlois et al., 2005; 2008), showing highest rates in nutrient depleted surface to subsurface waters (Großkopf et al., 2012).

We investigated the effect of variable nitrate and phosphate supply on phytoplankton growth and addressed the diazotrophic response to changes in N:P stoichiometry over time in two consecutive mesocosm experiments. In order to extend the design of previous mesocosm experiments (Franz et al., 2012), N and P supply ratios were varied while keeping either nitrate or phosphate at constant concentrations. High N:P ratios were applied to investigate potential inhibition of N_2 fixation, while low N:P supply ratios were applied to unravel the role of excess P and consecutively formed DOP on primary production and diazotrophy. Direct N_2 fixation rate measurements as well as determination of *nifH* gene and transcript abundances were carried out to characterize the diazotrophic community and their response to the chosen treatment levels. The experimental design and response variables were chosen in order to assess responses of the phytoplankton community to possible changes in oceanic nutrient stoichiometry as a consequence of ocean deoxygenation.

Methods

Experimental setup

In October 2012 we conducted two 8-day mesocosm experiments at the Instituto Nacional de Desenvolvimento das Pescas (INDP), Mindelo, Cabo Verde. The night before the start of each experiment, surface water was collected with RV *Islândia* south of São Vicente (16°44.4'N, 25°09.4'W) and transported to shore using four 600 L food safe intermediate bulk containers. Containers for water transport were first rinsed with diluted HCl and several times with deionized water. The experimental setup comprised 16 plastic mesocosm bags, which were distributed in four flow-through water baths. Blue, transparent lids were added to reduce the light intensity to approximately 20% of surface irradiation. The collected water was evenly distributed among mesocosm bags by gravity, using a submerged hose to minimize bubbles. The volume inside each mesocosm was calculated after adding 1.5 mmol silicic acid and measuring the resulting silicic acid concentration. The volume ranged from 105.5–145 liters. Nutrients in all mesocosms were measured before nutrient manipulation. Nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}) and silicic acid ($\text{Si}(\text{OH})_4$) were all below the detection limit and far below the manipulation levels (see Fig. 2). We therefore conclude that no contamination with these nutrients occurred during water sampling, transport and mesocosm filling. Experimental manipulation was achieved by adding different amounts of nitrate and phosphate. In the first experiment, the phosphate supply was changed at constant nitrate supply (*varied P*) in 13 of the 16 units, while in the second experiment the nitrate supply was changed at constant phosphate supply (*varied N*) in 12 of the 16 units. Each of these nutrient treatments was replicated 3 times. In addition to this, “cornerpoints” were chosen, where both the nitrate and phosphate supply was changed. The „cornerpoints“ were not replicated. These treatments were repeated during both experiments (see Fig. 1 for experimental design). Four cornerpoints should have been repeated, but due to erroneous nutrient levels in mesocosm 10 during *varied N*, this mesocosm also was adjusted to the center point conditions. Experimental treatments were randomly distributed between the four water baths. Initial sampling was carried out immediately after filling of the mesocosms on day 1.

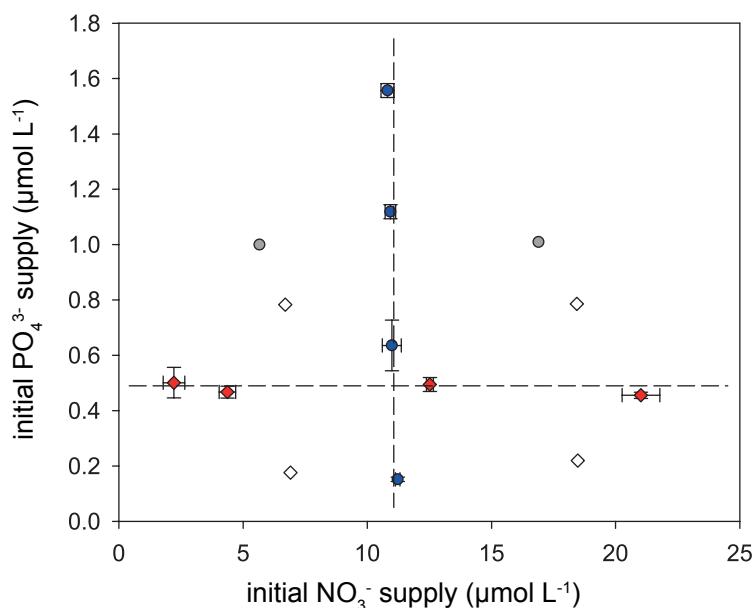


Figure 1: Experimental design and initial nutrient supply conditions during varied P (blue circles) and varied N (red diamonds). “Cornerpoints” during varied P and varied N are depicted as grey circles and white diamonds, respectively. Error bars denote the standard deviation of replicated ($n=3$) treatments.

After nutrient manipulation, sampling was conducted on a daily basis between 09:00 and 10:30 local time for days 2 to 8. Nutrient levels were set between 2 and 20 $\mu\text{mol L}^{-1}$ for nitrate, 0.25 and 1.75 $\mu\text{mol L}^{-1}$ for phosphate and 15 $\mu\text{mol L}^{-1}$ for silicic acid. Table S1 gives the target nutrient concentrations and corresponding measured concentrations in the mesocosms.

It has to be noted that no algal bloom developed in mesocosm 5 during *varied N* (target concentrations: 17.65 $\mu\text{mol L}^{-1}$ NO_3^- , 0.40 $\mu\text{mol L}^{-1}$ PO_4^{3-}). Thus, it was not included in the analysis and data are not presented.

Although we refer to our experimental approach as mesocosm experiment, this label might be disputable depending on the definition of the term mesocosm. Sometimes, experimental enclosures are only defined by size, where our approach would fall into the range of a microcosm experiment ($<1 \text{ m}^3$; Riebesell et al., 2010). Independent of its size, a mesocosm can also be defined as a confined body of water, where environmental factors are manipulated at the community or ecosystem level (Stewart et al., 2013). In contrast, microcosm experiments are often used to manipulate factors at the population level and often lack the realism to extrapolate results to natural systems (Stewart et al., 2013). Although our experimental enclosures are limited in size, we consider it justified using the term mesocosm, as we conducted our experiments with natural communities consisting of at least three trophic levels (bacteria, phytoplankton, microzooplankton).

Nutrients

Samples (10 mL) for dissolved inorganic nutrients (NO_3^- , NO_2^- , PO_4^{3-} , Si(OH)_4) were taken daily from each mesocosm and measured directly using a QuAatro Autoanalyzer (Seal Analytic) according to Grasshoff et al. (1999). The detection limits of nutrient analyses were $0.01 \mu\text{mol L}^{-1}$ for NO_2^- and PO_4^{3-} , $0.03 \mu\text{mol L}^{-1}$ for NO_3^- and $0.04 \mu\text{mol L}^{-1}$ for Si(OH)_4 .

Chlorophyll *a*

For chlorophyll *a* (Chl *a*) analyses, water samples (0.5–1 L) were vacuum-filtered (200 mbar) onto Whatman GF/F filters (25 mm, $0.7 \mu\text{m}$) before adding 1 mL of ultrapure water. Filters were immediately stored frozen for at least 24 hours. 9 mL acetone (100%) was then added to each sample and the fluorescence was measured with a Turner Trilogy fluorometer, which was calibrated with a Chl *a* standard dilution series (*Anacystis nidulans*, Walter CMP, Kiel, Germany). Chl *a* concentrations were determined according to Parsons et al. (1984).

Dissolved organic phosphorus

Water samples for analyses were filtered through pre-combusted (450°C , 5 hours) Whatman GF/F filters (25 mm, $0.7 \mu\text{m}$). The filtrate was stored in acid-clean 60 mL HDPE bottles (5% HCl for at least 12 hours) and frozen at -20°C until further analysis.

Prior to analysis of total dissolved phosphorus (TDP) one metering spoon of the oxidizing reagent Oxisolv (Merck) was added to 40 mL of sample, which was hereupon autoclaved for 30 minutes. Samples were then analyzed spectrophotometrically (Autoanalyzer QuAatro Seal Analytic), following Bran and Luebbe AutoAnalyzer Method No. G-175-96 Rev. 13 (PO_4^{3-}). The detection limit was $0.2 \mu\text{mol L}^{-1}$ and analytical precision was $\pm 8.3\%$.

DOP concentrations were calculated as:

$$\text{DOP} = \text{TDP} - \text{PO}_4^{3-} \quad (1)$$

Particulate organic matter

Particulate organic matter concentrations were determined by filtering 0.5–1 L seawater through pre-combusted (450°C for 5 hours) Whatman GF/F filters (25 mm, $0.7 \mu\text{m}$) under low pressure (200 mbar).

Filters were immediately frozen and stored until analysis.

Prior to analysis, particulate organic carbon (POC) and nitrogen (PON) filters were fumed with HCl (37%, for 24 hours) in order to remove inorganic carbon. After drying, filters were wrapped in tin cups ($8 \times 8 \times 15$ mm) and measured according to Sharp (1974) using an elemental analyzer (Euro EA, EuroVector, Milan, Italy).

For particulate organic phosphorus (POP) measurements, filters were autoclaved with the oxidation reagent Oxisolv (Merck) and 40 mL of ultrapure water for 30 min in a pressure cooker. Then, orthophosphate was analyzed photometrically according to Hansen and Koroleff (1999).

Relationships of dissolved and particulate organic matter accumulation to the inorganic nutrient supply ratios were determined using Model I regression analyses (SigmaPlot, Systat).

Molecular methods

Samples for the extraction of DNA/RNA were taken by filtering a volume of 1–2 L (exact volumes and filtration times were determined and recorded continuously) of seawater through 0.2 μ m polyethersulfon membrane filters (Millipore, Billerica, MA, USA). The filters were frozen and stored at -80°C until analysis. Nucleic acid extraction was performed using the Qiagen DNA/RNA All prep Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The extracted RNA was reverse transcribed to cDNA using the Superscript III First Strand synthesis Kit (Invitrogen) following the manufacturer's protocol with primers *nifH2* and *nifH3* (Langlois et al., 2005; Zani et al., 2000). *NifH* clusters were quantified from DNA and cDNA by quantitative Real Time PCRs as previously described by Church et al. (2005) and Langlois et al. (2008). TaqMan® qPCRs were set up in 12.5 μ l reactions and were performed in technical duplicates in an ABI ViiA7 qPCR system (Life technologies, Carlsbad, CA, USA). For each primer and probe set, standard curves were obtained from dilution series ranging from 10^7 to 10 gene copies per reaction; standards were constructed using plasmids containing the target *nifH* gene. Sequences of primers and probes are given in Table 1. To confirm purity of RNA, non-template qPCRs were performed using the corresponding RNA.

Table 1: Primers and probes used in *nifH* TaqMan qPCR assays.

Target Group	Reverse primer (5'-3')	Forward Primer (5'-3')	Probe (5'-3')
Filamentous	GCAAATCCACCGCAAAC AAC	TGGCCGTGGTATTATTA CTGCTATC	AAGGAGCTTATACAGAT CTA
UCYN-A	TCAGGACCACCGGACTC AAC	TAGCTGCAGAAAGAGGA ACTGTAGAAG	TAATTCCTGGCTATAAC AAC
UCYN-B	TCAGGACCACCAGATTC TACACACT	TGCTGAAATGGGTTCCTG TTGAA	CGAAGACGTAATGCTC
UCYN-C	GGTATCCTTCAAGTAGT ACTTCGTCTAGCT	TCTACCCGTTTGATGCT ACACACTAA	AAACTACCATTCTTCAC TTAGCAG
GamAO	AACAATGTAGATTTCTCT GAGCCTTATTC	TTATGATGTTCTAGGTG ATGTG	TTGCAATGCCTATTTCG
Het I	AATACCACGACCCGCAC AAC	CGGTTTCCGTGGTGTAC GTT	TCCGGTGGTCCTGAGCC TGGTGT
Het II	AATGCCGCGACCAGCAC AAC	TGGTTACCGTGATGTAC GTT	TCTGGTGGTCCTGAGCC TGGTGT

¹⁵N₂ seawater incubations

Seawater incubations were performed in triplicates from each mesocosm on day 1 and day 8 of both experiments as previously described by Mohr et al. (2010) and Großkopf et al. (2012). Degassed seawater was filled into evacuated gas-tight 3 L Tedlar[®] bags without a headspace. Addition of ¹⁵N₂ gas was (depending on the exact water volume in the Tedlar[®] bag) around 10 mL ¹⁵N₂ per 1 L seawater. Dissolution of the ¹⁵N₂ gas was achieved by ‘slapping’ the bubble with a ruler. After complete dissolution of the added ¹⁵N₂ gas (¹⁵N₂-enriched seawater), an aliquot of the ¹⁵N₂ enriched water was collected for each preparation of enriched seawater and stored in an Exetainer. Seawater samples were filled headspace-free; 100 mL of seawater was exchanged with previously degassed seawater containing a defined concentration ¹⁵N₂ and ¹³C-NaCO₃. Incubations were performed in 4.5 L polycarbonate bottles closed with Teflon[®]-coated butyl rubber septum caps. The ¹⁵N₂ concentration in the prepared batches of enriched water was determined to be 250 µmol L⁻¹, which translates in an ¹⁵N-enrichment of about 2% in the 4.5 L bottle incubations, when adding 100 mL enriched seawater (depending on temperature and salinity). Water samples were incubated for 24 hours in the mesocosm water

baths, thus at the same temperature and light regime, followed by a filtration on Whatman GF/F filters, which were analyzed using mass spectrometry as previously described in Loescher et al. (2014).

Results

Bloom development and nutrient dynamics in the mesocosms

In both consecutive experiments (*varied P* and *N*) a bloom formation was observed following nutrient manipulation. Nitrate and phosphate were readily taken up by the plankton community and nutrient concentrations thus declined until the end of the experiment (Fig. 2). NO_3^- was fully depleted in all mesocosms at days 6–8 in both runs, except in the mesocosms with highest N:P ratios of 48:1 (treatment 12.00N/0.25P in *varied P*) and 44:1 (treatment 17.65N/0.40P in *varied N*). Residual PO_4^{3-} was still detectable at the end of the experiments (day 8) in all mesocosms with initial N:P values <10 (treatments in *varied P*: 6.35N/1.10P, 12.00N/1.25P, 12.00N/1.75P; treatments in *varied N*: 2.00N/0.75P, 4.00N/0.75P, 6.00N/1.03P) indicating a limitation of primary productivity dependent on the N:P ratio.

Although initial Chl *a* concentrations were slightly higher in *varied P* than in *varied N* ($\sim 0.38 \mu\text{g L}^{-1}$ and $0.2 \mu\text{g L}^{-1}$, respectively), the increase in Chl *a* concentration was 5–10-fold until days 5/6 in *varied P* compared to 10–50-fold in *varied N*. After the bloom at days 5 and 6 Chl *a* declined again to $0.05\text{--}0.7 \mu\text{g L}^{-1}$ and $0.6\text{--}1.7 \mu\text{g L}^{-1}$ in *varied P* and *varied N*, respectively (Fig. 2).

Particulate organic matter (POM) accumulation and stoichiometry

Temporal dynamics of POM were similar during both experiments. Initial concentrations of POC, PON and POP were $10\text{--}17 \mu\text{mol L}^{-1}$, $1.5\text{--}2 \mu\text{mol L}^{-1}$ and $0.05\text{--}0.12 \mu\text{mol L}^{-1}$, respectively (Fig. 2). In *varied P*, POC and PON reached a maximum on day 6, while POP increased until the end of the experiment.

In *varied N* POM accumulation also peaked on day 6 or 7 in most mesocosms, but differences between N:P treatments were more pronounced in *varied N* compared to *varied P*. Our results indicate that POM accumulation was independent of the initial nutrient supply ratio in both experiments (Fig. 3). We observed a significantly positive regression coefficient between maximum POC and PON concentrations (defined as peak POC and PON concentration subtracted by the initial (day 1) POC and PON concentration) to the initial NO_3^- supply (POC: $r^2 = 0.64$, $p = 0.0006$; PON: $r^2 = 0.80$, $p < 0.0001$) while POP accumulation showed a significantly positive regression coefficient to initial PO_4^{3-} supply ($r^2 = 0.31$, $p = 0.048$).

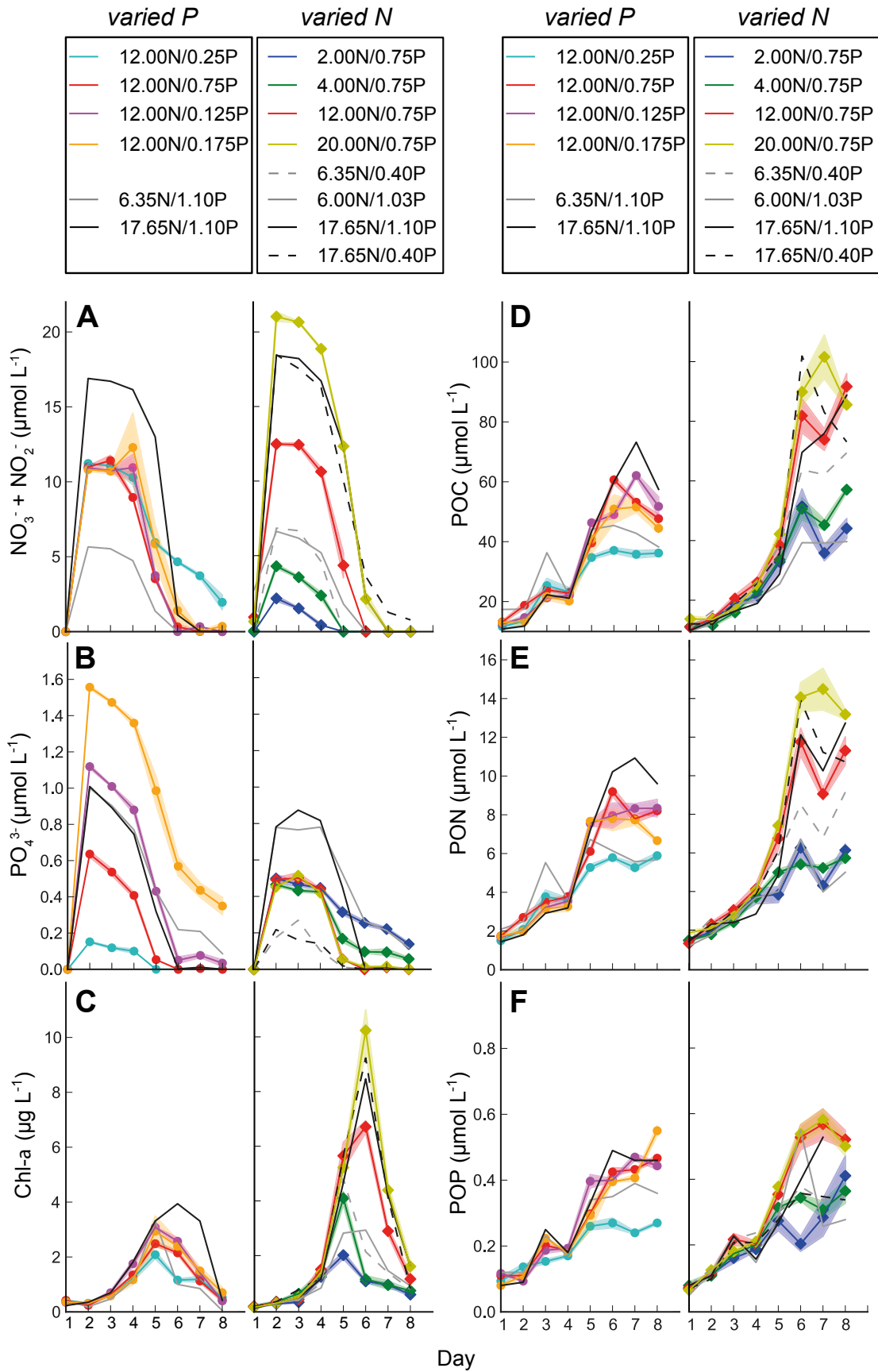


Figure 2: Temporal development of (A) NO₃⁻ and NO₂⁻, (B) PO₄³⁻, (C) Chl a, (D) POC, (E) PON and (F) POP within all treatments of both experimental runs. Standard deviations are depicted as shaded error bands.

Mean PON:POP ratios during the exponential growth phase appeared to be independent of the initial N:P supply ratio in both experimental runs (Fig. 4). With ratios between 17 and 23, the PON:POP ratios were above, but close to Redfield proportion in all treatments during the first 5 days of the experiments, consistent with an observed initial uptake of N:P in Redfield proportions in all mesocosms. During the post bloom phase, mean PON:POP ratios were positively correlated with the initial nutrient supply ratio ($r^2 = 0.73$, $p < 0.0001$). Nevertheless, stoichiometry of POM (N:P between 16 and 32) exceeded Redfield proportions, even in treatments with lowest N:P ratios.

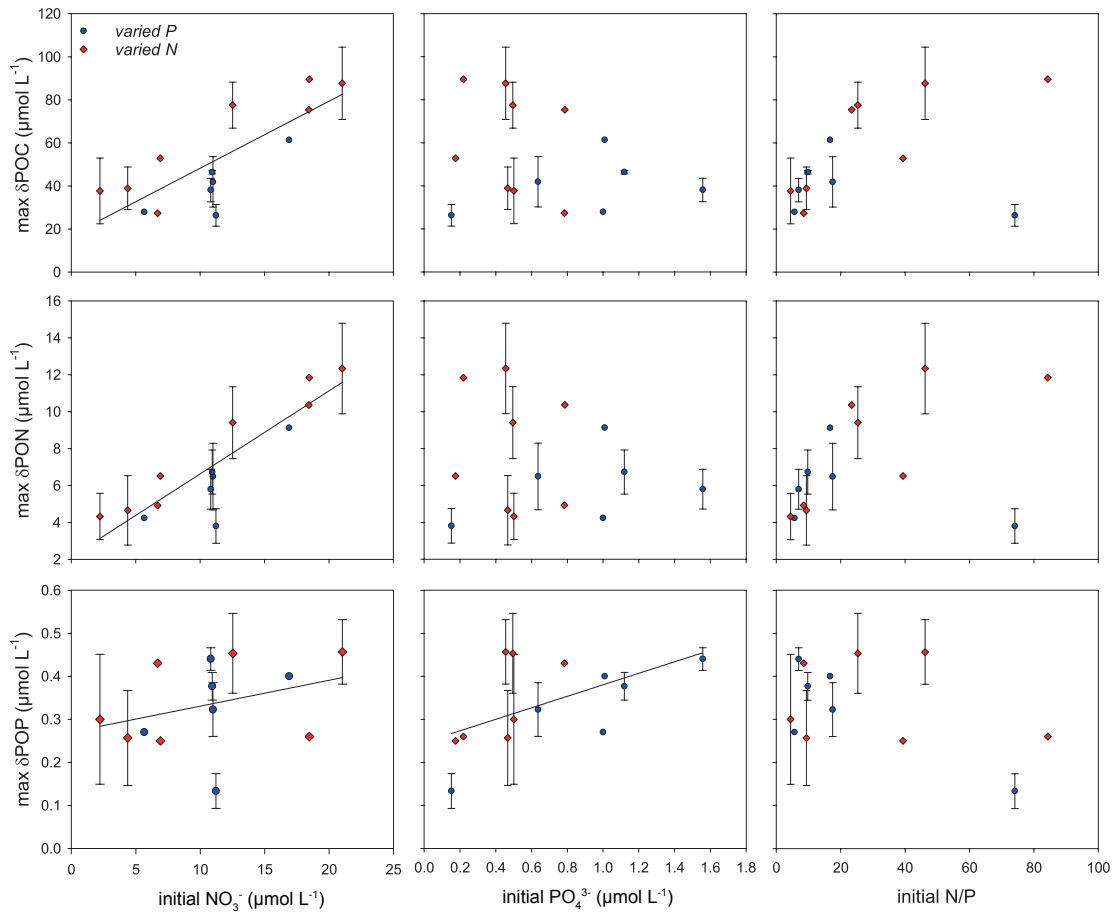


Figure 3: Maximum POC, PON and POP accumulation as a function of the initial supply of NO_3^- , PO_4^{3-} and N:P. Maximum δPOM is defined as peak POM concentration subtracted by the initial (day 1) POM concentration. Treatments in varied P are depicted as blue circles; treatments in varied N are depicted as red diamonds. Error bars denote the standard deviation of replicated ($n=3$) treatments. Regression lines (continuous lines) indicate significant linear correlations between the initial nutrient supply and POM accumulation.

Dissolved organic phosphorus dynamics

Initial DOP concentrations during varied P were $0.14 (\pm 0.009) \mu\text{mol L}^{-1}$. In most mesocosms, except for the one with lowest initial PO_4^{3-} supply (12.00N/0.25P), DOP concentrations increased progressively until the end of the experiment (Fig. 5). Highest DOP concentrations of around $0.4 \mu\text{mol L}^{-1}$ were determined in mesocosm 12.00N/0.75P on day 5 and decreased again afterwards. Maximum DOP accumulation (defined as described for maximum POM accumulation, section 3.2) was significantly correlated to the initial PO_4^{3-} supply (Fig. 6; $r^2 = 0.63$, $p = 0.0007$).

In *varied N* initial DOP concentrations in the mesocosms were $0.2 (\pm 0.038) \mu\text{mol L}^{-1}$ and increased slightly until day 3. Afterwards DOP concentrations remained rather constant, although with considerable variability in the data (Fig. 5).

A simple mass balance (Table S2) showed that part of the phosphorus pool, i.e. the sum of PO_4^{3-} , DOP and POP, remained unaccounted for (P pool_x) at the end of the experiment (P pool_x in *varied P* ~25% of the initial P pool, P pool_x in *varied N* ~14%). This undetermined P pool is most likely due to wall growth, which became visible towards the end of the experiment. However, only in two mesocosms the difference between P pools sizes on day 2 and day 8 was significant.

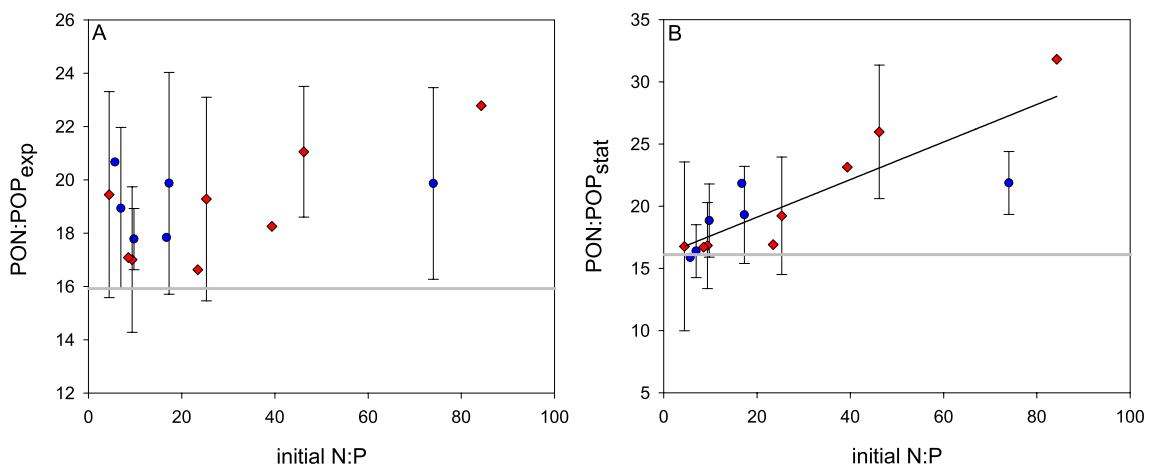


Figure 4: PON:POP stoichiometry during (A) the exponential growth phase and (B) the stationary growth phase of the experiment. The grey line visualizes the Redfield Ratio. The color code, symbols and lines are the same as in Fig. 3.

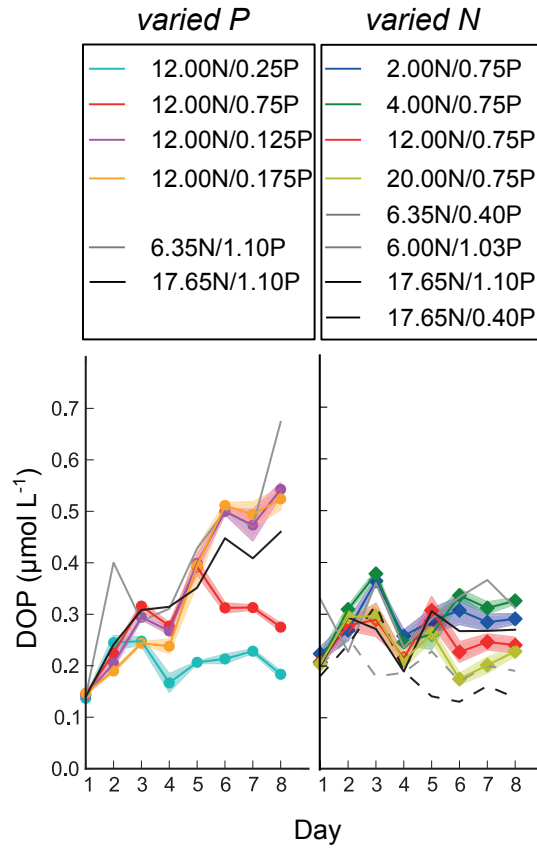


Figure 5: Temporal development of DOP with standard deviations depicted as shaded error bands.

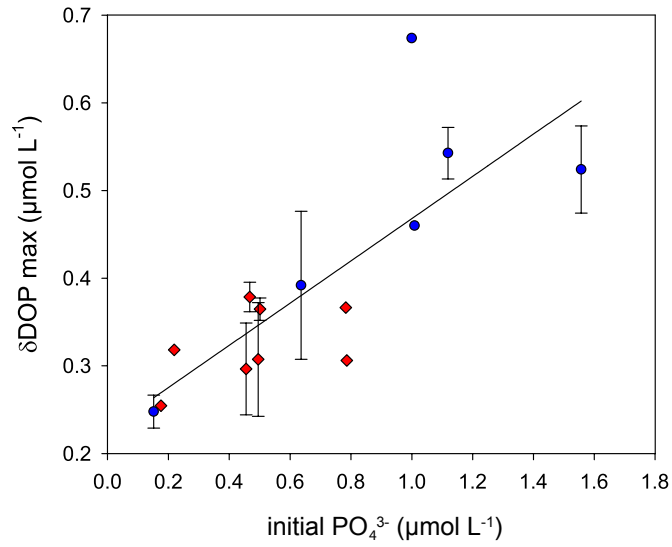


Figure 6: Positive linear correlation between maximum DOP accumulation (defined as peak DOP concentration subtracted by the initial DOP concentration) and initial PO_4^{3-} supply during varied P (blue circles) and varied N (red diamonds).

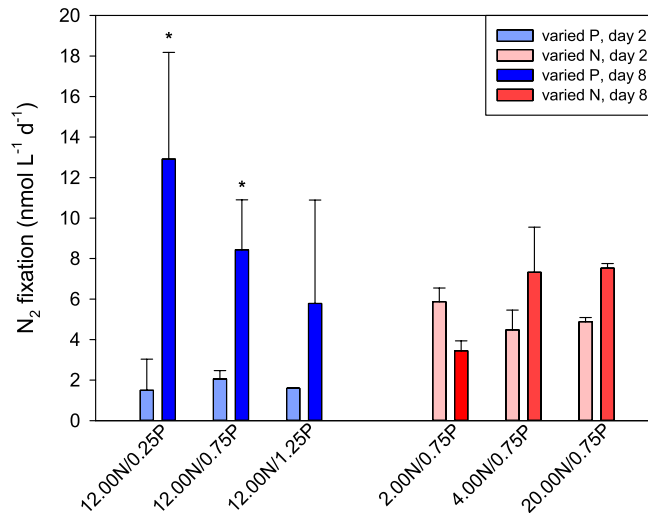


Figure 7: Mean N_2 fixation rates measured on day 2 and day 8 of both experiments. Because of the high variance between replicates we omitted N_2 fixation rates from un-replicated treatments. Asterisks indicate a significant difference between day 2 and day 8 (t -test). Error bars indicate the standard deviation.

Importance of the *Richelia-Rhizosolenia* symbiosis for diazotrophy

Directly measured rates of N_2 fixation showed an increase with time in *varied P*, while no statistically significant increase could be observed in *varied N* (Fig. 7). A molecular screening of the diazotrophic community in the initial water batch used for *varied P* using the *nifH* gene as functional marker gene showed a dominance of filamentous cyanobacterial diazotrophs related to *Trichodesmium* accounting for ~54% of the diazotrophic community (results from qPCR), followed by proteobacterial diazotrophs (~36%) in *varied P* (data not shown).

The high abundance of filamentous cyanobacterial diazotrophs indicated the presence of a bloom in the initial water batch in *varied P*. In *varied N*, the initial community consisted mainly of proteobacterial diazotrophs (~88%), followed by UCYN-B (9%) and filamentous cyanobacteria (3%).

Changes in transcript abundance over time were most intense for *Richelia-Rhizosolenia* (Het I) transcripts (Fig. 8). At day 2, Het I transcript abundances were higher in *varied N* conditions compared to *varied P*. This relation changed over the course of the experiments, with a pronounced increase of Het I transcript abundances between day 6 and 8 in *varied P*. Thus, all classical *nifH* clusters (filamentous cyanobacteria, UCYN-A, -B, -C and proteobacteria diazotrophs) decreased in abundance of genes and gene transcripts down to the

detection limit in both experiments, whereas diazotrophs of the *Richelia-Rhizosolenia* symbiosis were the only diazotrophs that showed an increase in *nifH* transcripts over the course of the experiment, exclusively in *varied P* (Fig. 8). During *varied N*, *nifH* gene and transcript abundance of the *Richelia-Rhizosolenia* cluster was close to the detection limit and DOP accumulation was rather negligible. In contrast, we observed an accumulation of DOP in *varied P*. Here, mesocosms with a significant increase in N_2 fixation (12.00N/0.25P and 12.00N/0.75P) were also the ones where DOP was used as phosphorus-source for biomass build-up after PO_4^{3-} was depleted (Fig. 9).

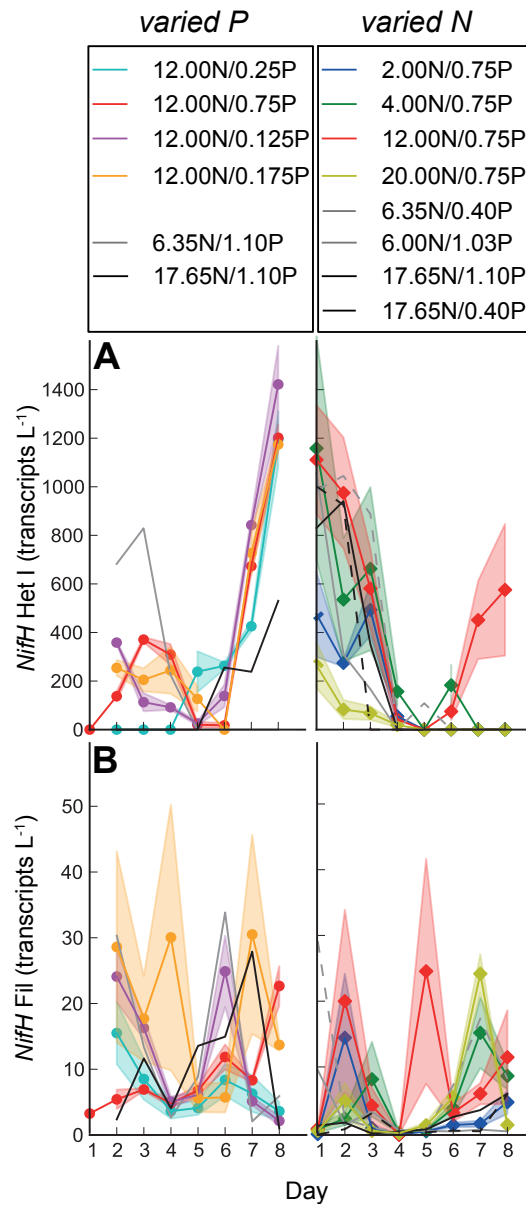


Figure 8: Temporal development of transcript abundances for (A) *Richelia-Rhizosolenia* (Het I) and filamentous cyanobacteria related to *Trichodesmium* (Fil). Standard deviations are depicted as shaded error bands.

In mesocosm 12.00N/0.75P, PO_4^{3-} concentrations were below the detection limit after day 5. This coincided with a decrease of DOP after day 5, while POP concentrations increased until the end of the experiment.

In mesocosm 12.00N/0.25P, POP also increased beyond the point of PO_4^{3-} depletion and highest POP accumulation exceeded values that could be explained by PO_4^{3-} incorporation alone. Thus a potential impact of DOP on diazotrophy is hypothesized. In mesocosms without a significant increase in N_2 fixation, POP and DOP concentrations increased until the end of the experiment and no apparent uptake of DOP could be observed.

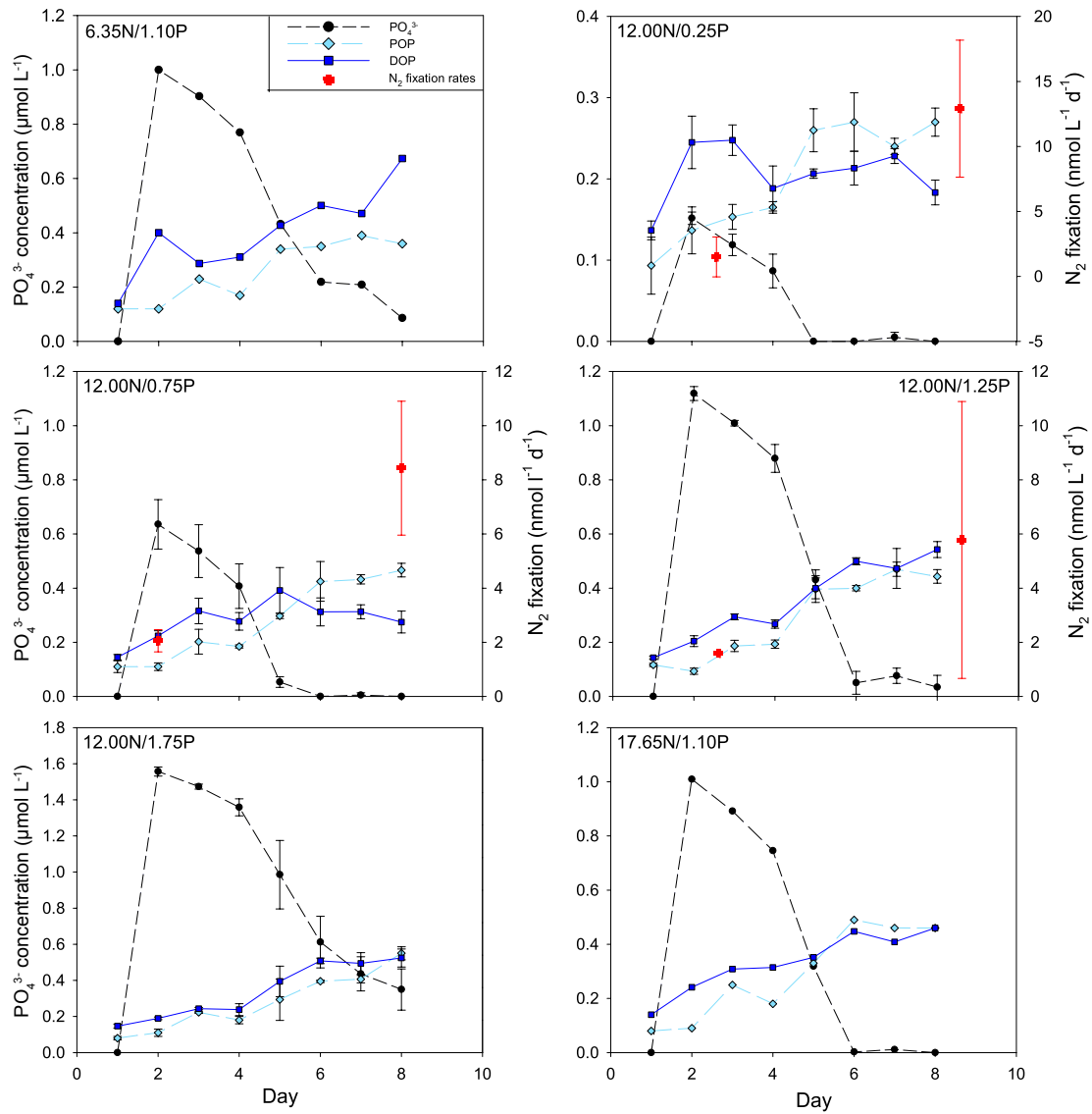


Figure 9: Dynamics of PO_4^{3-} , POP and DOP and N_2 fixation rates in mesocosms during varied P. Because of the high variance between replicates we omitted N_2 fixation rates from un-replicated treatment.

Discussion

Controls on plankton production

In order to understand potential consequences of changes in nutrient regimes, it is necessary to determine the factors that control and limit microbial production. In our experiments, amendments of NO_3^- significantly increased chlorophyll concentrations and enhanced the accumulation of POM, indicating the ability of the plankton community to rapidly and intensively react to nitrate availability. These results suggest that the ultimate limiting nutrient for phytoplankton production in our experiment was NO_3^- . N_2 fixation was measurable in all initial samples, which indicates the presence of a niche for diazotrophs in the Cabo Verde region. For the upwelling region as well as for the oligotrophic open ocean of the ETNA, nitrate limitation of the phytoplankton community has previously been reported (Davey et al., 2008; Franz et al., 2012; Moore et al., 2008). Additionally, Moore et al. (2008) observed a co-limitation of nitrate and phosphate during nutrient addition bioassay experiments in the ETNA. In our experiment, however, only POP accumulation was positively affected by PO_4^{3-} supply. This argues against a secondary limitation by phosphate, but rather points towards a mechanism of accumulating and storing phosphate as polyphosphate within the cell (Geider and La Roche, 2002; Martin et al., 2014; Schelske and Sicko-Goad, 1990).

There is a large difference between the supply ratio of inorganic nutrients and the PON:POP ratio of the plankton community in our study. Although initial N:P ratios in our mesocosms covered a wide range, PON:POP ratios reached maximum values of ~ 21 in both experiments during the exponential growth phase. During stationary growth, maximum PON:POP values of 39 in *varied N* and 22 in *varied P* were measured. However, during growth phases in both experiments PON:POP ratios never fell below 16. Very similar results were obtained by Franz et al. (2012) off the Peruvian coast. However, two experiments conducted by Franz et al. (2012) in the ETNA and off West Africa showed a different response of the phytoplankton community. In these two cases, N:P supply ratio and PON:POP were highly correlated and PON:POP ratios as low as $6.0 (\pm 1.4)$ were observed in the stagnant phase. This shows that the stoichiometry of phytoplankton communities is flexible to a certain extent, but does not necessarily reach dimensions observed in laboratory experiments (Hecky et al., 1993) and implied by theoretical approaches (e.g.

Geider and La Roche, 2002; Klausmeier et al., 2004). This may result from differences in the initial community composition if it lacks organisms able to assemble a P rich growth machinery (Arrigo, 2005; Klausmeier et al., 2004). It has been reported that cellular N content seems relatively inflexible in some phytoplankton groups, thus restricting the maintenance of metabolic processes at low dissolved inorganic nitrogen concentrations (Moore et al., 2013). In contrast, phosphate requirements seem to be comparably flexible, as certain cellular components containing P (e.g. phospholipids) can be replaced by non-phosphorus containing compounds (Moore et al., 2013). This can also be deduced from our experiments, where higher N:P ratios lead to increasing PON:POP ratios, possibly due to the flexibility to substitute P compounds within the biomass. In contrast, lower N:P ratios lead to lower biomass accumulation, as the plasticity of PON:POP seems to be constrained by the availability of nitrate in our experiments.

The impact of bioavailable N on N₂ fixation

The ability of diazotrophs to grow independent of a fixed N source in principle gives them an advantage to thrive under conditions where their competitors are limited by N availability. At the same time, diazotrophs are considered disadvantaged when competing with faster growing non-diazotrophs for nutrients under N replete conditions (Tyrrell, 1999; Ward et al., 2013). Contrary to this classical view, we could not detect a direct influence of reactive N compounds on N₂ fixation in our experiments. Despite a wide spectrum of applied nitrate concentrations in *varied N*, no significant difference in N₂ fixation rates could be detected. Evidence from culture experiments also suggests that inorganic N compounds do not always repress N₂ fixation. While NO₃⁻ addition in *Trichodesmium* spp. (Holl and Montoya, 2005; Mulholland et al., 2001) and NH₄⁺ addition in *Crocospaera watsonii* (Dekaezemacker and Bonnet, 2011) reduced N₂ fixation rates, NO₃⁻ addition did not reduce N₂ fixation rates in *C. watsonii* and *Nodularia* spp. cultures (Dekaezemacker and Bonnet, 2011; Sanz-Alférez and del Campo, 1994). Moreover, recent field surveys demonstrated the occurrence of N₂ fixation in nutrient rich water masses of the eastern tropical South Pacific (ETSP) and equatorial Atlantic upwelling regions (Fernandez et al., 2011; Loescher et al., 2014; Subramaniam et al., 2013) and also modeling studies predict high N₂ fixation rates in waters containing measurable amounts of reactive N (Deutsch et al., 2012; Weber and Deutsch, 2014). Clearly, the degree of feedback

concerning the inhibition of N_2 fixation by reactive N compounds is not universal and there is evidence that the absence of P and Fe in seawater is a stronger indicator for limitation of N_2 fixation than the presence of inorganic N compounds (Weber and Deutsch, 2014).

The role of excess P and DOP as controls on N_2 fixation

Deutsch et al. (2007) suggested that N_2 fixation is favored in upwelling regions, where N loss in adjacent OMZ waters and P leaching from the sediment lead to upwelling of waters enriched in P. This excess P is thought to be consumed by diazotrophs, thus replenishing the N-deficit in the vicinity of upwelling regions.

As nutrients were taken up in Redfield or above Redfield proportions in our experiments we would have expected excess phosphate in mesocosms with N:P supply ratios below Redfield. Instead, excess phosphate was absent and our data point towards a channeling of PO_4^{3-} through the particulate pool into DOP, as an increase in PO_4^{3-} supply significantly increased the concentration of DOP. Why phytoplankton synthesize and excrete higher levels of DOP under excess phosphate conditions remains unclear, but enhanced PO_4^{3-} uptake (followed by DOP accumulation) is thought to hamper P limitation when sudden boosts in N are encountered (Mackey et al., 2012). In accordance with our study, mesocosm experiments from the ETNA and eastern tropical south Pacific (ETSP) open ocean (Franz et al., 2012) and measurements from shelf regions of the ETNA (Reynolds et al., 2014) and Celtic Sea (Davis et al., 2014) showed the accumulation of DOP under excess phosphate supply. Although the composition and bioavailability of the DOP pool needs to be further evaluated, DOP may act as a source of P for prokaryotic primary producers, either exclusively or in addition to PO_4^{3-} (Björkman and Karl, 2003; Dyhrman et al., 2006; Mahaffey et al., 2014; Reynolds et al., 2014). This indicates that the ability to utilize DOP may give diazotrophs a competitive advantage when bioavailable forms of N are depleted and either PO_4^{3-} or DOP concentrations are sufficient.

In our experiments a significant increase in N_2 fixation rates was only detected in *varied P*. In mesocosms with highest N_2 fixation rates, PO_4^{3-} was depleted after day 5 or 6 while POP increased until the end of the experiment. After PO_4^{3-} depletion, DOP concentrations declined, which indicates that DOP served as phosphorus source until the end of the experiment. It has to be noted that N_2 fixation rates were only measured at the beginning and the end of our

experiment and possible fluctuations over time cannot be accounted for. However, increasing diazotrophic transcript abundances of *Richelia intracellularis* in symbiosis with the diatom *Rhizosolenia* (Het I) were also detected over the course of the *varied P* experiment. While the diatom abundance was probably favored by replete amounts of silicic acid added at the beginning of the experiment, no increase in diatom-diazotroph associations (DDAs) was detected in the *varied N* experiment. Measured N₂ fixation rates and transcript abundances lead us to speculate that DDAs were favored in the *varied P* experiment, where diazotrophs in the mesocosms utilized DOP resources in order to supply P to themselves and/or their symbiont. The ability to utilize DOP has previously been shown for *R. intracellularis* (Girault et al., 2013) and our observations suggest that they may not only provide their symbionts with N via N₂ fixation but also with P via DOP utilization.

DDAs in our experiment were favored by replete amounts of silicic acid and DOP and were – in contrast to the classical view – not restrained by reactive N compounds. These findings suggest that DDAs have the potential to actively fix nitrogen in shelf waters of upwelling regions. Therefore, the N-deficit of upwelled water-masses could already be replenished locally prior to offshore transport.

A shift within the diazotrophic community towards DDAs could also exert controls on carbon export. Grazing, particle aggregation and export likely increase when filamentous and proteobacterial cyanobacteria are replaced by DDAs (e.g. Berthelot et al., 2015; Karl and Letelier, 2008; Karl et al., 2012). The enhanced strength and efficiency of the biological pump would therefore increase the potential for carbon sequestration in the ETNA.

Conclusions and future implications for the ETNA

Our findings add to the growing evidence that diminished N:P ratios in upwelling waters in the ETNA will either decrease the biomass of non-diazotrophic primary producers, specifically due to the decline of bioavailable N, or lead to a community shift towards primary producers that are able to adapt to changing N:P conditions. As a considerable amount of DOP was produced under excess phosphate conditions, changes in the N:P ratio of waters could exert profound control over DOP production rates in the ETNA. Our results indicate that enhanced DOP production in upwelling regions will likely fuel N₂ fixation, with an advantage for those diazotrophs capable of DOP utilization. We propose that N₂ fixation in the ETNA might not only be restricted to the oligotrophic open ocean but can occur in nutrient-rich upwelling regions as previously demonstrated for the tropical Pacific (Löscher et al., 2014) and the Atlantic equatorial upwelling (Subramanian et al., 2013), as N₂ fixation in DDAs seems to be favored by the presence of silicic acid and DOP, and not by the absence of fixed N compounds.

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All data were uploaded to www.pangaea.de after publication.

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Appendix

Table S1: Nominal and measured nutrient concentrations after the addition of nitrate or phosphate to the mesocosms in both experimental runs.

Run	Treat ID	NO ₃ ⁻ nom [μmol L ⁻¹]	PO ₄ ³⁻ nom [μmol L ⁻¹]	SiO ₂ nom [μmol L ⁻¹]	N : P nom	NO ₃ ⁻ [μmol L ⁻¹]	PO ₄ ³⁻ [μmol L ⁻¹]	SiO ₂ [μmol L ⁻¹]	N : P
1	12.0N/0.75P	12	0.75	15	16	11.52	0.73	15.22	15.78
1	12.0N/0.75P	12	0.75	15	16	10.97	0.68	14.97	16.11
1	12.0N/0.75P	12	0.75	15	16	10.63	0.52	15.04	20.47
1	6.35N/1.10P	6.35	1.1	15	5.76	5.66	1.00	15.06	5.66
1	12.0N/1.25P	12	1.25	15	9.6	10.74	1.14	15.01	9.39
1	12.0N/1.25P	12	1.25	15	9.6	11.16	1.12	15.33	9.95
1	12.0N/1.25P	12	1.25	15	9.6	10.89	1.09	15.13	9.97
1	12.0N/1.75P	12	1.75	15	6.86	10.55	1.57	14.97	6.74
1	12.0N/0.75P	12	0.75	15	16	10.82	0.61	15.10	17.64
1	12.0N/1.75P	12	1.75	15	6.86	10.82	1.58	14.90	6.86
1	12.0N/1.75P	12	1.75	15	6.86	11.07	1.53	15.01	7.24
1	12.0N/0.25P	12	0.25	15	48	11.16	0.15	15.12	76.78
1	12.0N/0.25P	12	0.25	15	48	11.18	0.16	15.00	69.80
1	17.65N/1.10P	17.65	1.1	15	16	16.90	1.01	15.27	16.75
1	12.0N/0.25P	12	0.25	15	48	11.33	0.15	15.15	75.77
2	12.0N/0.75P	12	0.75	15	16	12.58	0.47	14.51	27.00
2	12.0N/0.75P	12	0.75	15	16	12.36	0.51	14.18	24.32
2	12.0N/0.75P	12	0.75	15	16	12.61	0.51	14.34	24.72
2	6.35N/0.40P	6.35	0.4	15	15.99	6.91	0.18	14.63	39.35
2	17.65N/1.10P	17.65	1.1	15	16.05	18.43	0.79	14.47	23.45
2	20.0N/0.75P	20	0.75	15	26.67	20.57	0.47	15.09	43.92
2	20.0N/0.75P	20	0.75	15	26.67	20.60	0.45	14.16	45.92
2	20.0N/0.75P	20	0.75	15	26.67	21.90	0.45	15.18	48.81
2	4.00N/0.75P	4	0.75	15	5.33	4.62	0.45	15.33	10.38
2	17.65N/0.40P	17.65	0.4	15	44.46	18.47	0.22	15.36	84.31
2	4.00N/0.75P	4	0.75	15	5.33	4.49	0.47	14.92	9.59
2	4.00N/0.75P	4	0.75	15	5.33	3.99	0.49	15.68	8.17
2	2.00N/0.75P	2	0.75	15	2.67	2.06	0.46	16.39	4.52
2	6.00N/1.03P	6.00	1.03	15	5.77	6.69	0.78	15.46	8.55
2	2.00N/0.75P	2	0.75	15	2.67	1.87	0.56	17.64	3.33
2	2.00N/0.75P	2	0.75	15	2.67	2.71	0.48	15.04	5.60

Table S2: Mass balance. $P \text{ pool} = PO_4^{3-} + DOP + POP$. $P \text{ pool}_x = \text{undetermined } P \text{ pool}$, which represents a combination of measurement errors and wall growth. Statistical significance (t -test) between pool sizes on day 2 and day 8 is denoted by asterisks. No t -tests were conducted when only one replicate was available.

Run	Treat ID	Replicates	P pool day 2 [μmol L ⁻¹]	P pool day 8 [μmol L ⁻¹]	P pool _x [μmol L ⁻¹]	P pool _x [%]
1	6.35N/1.10P	1	1.52	1.12	-0.40	26
1	12.0N/0.25P	3	0.53 ± 0.07	0.45 ± 0.03	-0.08	15
1	12.0N/0.75P	4	0.97 ± 0.12	0.71 ± 0.11	-0.26	27
1	12.0N/1.25P	3	1.42 ± 0.06	1.02 ± 0.10	-0.40*	28
1	12.0N/1.75P	3	1.86 ± 0.05	1.42 ± 0.20	-0.44*	24
1	17.65N/1.10P	1	1.34	0.92	-0.42	31
2	2.00N/0.75P	3	0.88 ± 0.11	0.84 ± 0.19	-0.04	5
2	4.00N/0.75P	3	0.89 ± 0.06	0.75 ± 0.12	-0.14	16
2	6.00N/1.03P	1	1.13	0.71	-0.42	37
2	6.35N/0.40P	1	0.56	0.54	-0.02	4
2	12.0N/0.75P	3	0.89 ± 0.07	0.76 ± 0.10	-0.13	15
2	17.65/0.40P	1	0.56	0.48	-0.08	14
2	17.65N/1.10P	1	1.19	1.15	-0.04	3
2	20.0N/0.75P	3	0.88 ± 0.03	0.73 ± 0.10	-0.15	17

The effect of nutrient stoichiometry on organic matter dynamics, phytoplankton community composition and diazotrophy in the eastern tropical South Pacific

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Abstract

Water masses influenced by oxygen minimum zones (OMZ) feature low inorganic nitrogen (N) to phosphorus (P) ratios. The surplus of P over N is thought to favor non-Redfield primary production by phytoplankton species adapted to exponential growth. Additionally, excess phosphate (P^*) is thought to provide a niche for nitrogen fixing organisms. In order to assess the effect of low inorganic nutrient ratios on the stoichiometry and composition of primary producers, biogeochemical measurements were carried out in 2012 during a research cruise in the eastern tropical South Pacific (ETSP). A succession of different phytoplankton functional groups was observed along onshore – offshore transects with diatoms dominating the productive upwelling region, and prymnesiophytes, cryptophytes and *Synechococcus* prevailing in the oligotrophic open ocean. Although inorganic nutrient supply ratios were below Redfield proportions throughout the sampling area, the stoichiometry of particulate organic nitrogen to phosphorus (PON:POP) generally exceeded ratios of 16:1. Despite $\text{PON:POP} \geq 16$, high P^* values in the surface layer (0–50 m) above the shelf rapidly decreased as water masses were advected beyond the shelf. There are three mechanisms which can explain these observations: (1) non-Redfield primary production, where the excess phosphorus in the biomass is directly released as dissolved organic phosphorus (DOP), (2) non-Redfield primary production, which is masked by a particulate organic matter pool mainly consisting of P depleted detrital biomass and/or (3) Redfield primary production combined with dinitrogen (N_2) fixation. Indirect evidence suggests that the three processes occur simultaneously in our study area; quantifying the relative importance of each of these mechanisms needs further investigation. Therefore, it remains uncertain whether the ETSP is a net sink for bioavailable N or whether the N-deficit in this area is replenished locally.

Introduction

The Humboldt Current system is one of four major eastern boundary upwelling systems (EBUS). It is characterized by intense, year-round upwelling of nutrient loaded waters that facilitate intense biological production in the euphotic zone (Pennington et al., 2006). Closely linked to the productive surface layer is an oxygen minimum zone (OMZ), where nitrogen (N) loss processes (denitrification and anammox) diminish the amount of bioavailable N (Goering, 1968; Hamersley et al., 2007). Hypoxia and anoxia induced phosphate (P) release from the sediment (Ingall and Jahnke, 1994; Lomnitz et al., 2015; Noffke et al., 2007) results in a surplus of P over N in the water column (referred to as P^* , after Deutsch et al., 2007). Thus, upwelled water masses feature N:P stoichiometries below the Redfield ratio, which describes the globally integrated ratio of macronutrients in seawater and in organic matter (C:N:P = 106:16:1, Redfield, 1958). The deficit of nitrate over phosphate is thought to create an environment beneficial for autotrophic nitrogen fixers (Deutsch et al., 2007), suggesting a close spatial coupling of N loss and N_2 fixation. The presumption is, that phytoplankton in the highly productive shelf area consume N and P in Redfield proportions, while not altering P^* as waters are transported offshore. It has been further proposed that high supplies of dissolved organic phosphorus (DOP), which are produced under excess P (Meyer et al., 2016; Ruttenberg and Dyhrman, 2012), might additionally stimulate growth of N_2 fixing diazotrophs (Franz et al., 2012b; Somes and Oschlies, 2015), as these organisms are known to use DOP as P source either exclusively or in addition to P (Dyhrman et al., 2006; Sohm and Capone, 2006). But despite low N:P ratios accompanied by replete amounts of P^* and DOP in upwelled waters, no evidence for a significant abundance of diazotrophic cyanobacteria or autotrophic N_2 fixation has yet been found in the Peruvian or Chilean upwelling systems (Dekaezemacker et al., 2013; Franz et al., 2012a; 2012b). However, N:P ratios in the surface layer are apparently restored to Redfield proportions and P^* values are reduced as water masses are advected offshore (Franz et al., 2012b). Non-Redfield utilization of inorganic nutrients has been suggested as an alternative pathway for the consumption of P^* (Arrigo, 2005; Franz et al., 2012b; Mills and Arrigo, 2010). Different N:P utilization ratios in phytoplankton have been confirmed by laboratory and field data, which vary with growth rate, taxonomy and nutrient availability (e.g. Geider and La Roche, 2002; Moore et al., 2008; Quigg et al.,

2003). Fast growing phytoplankton, for example, often utilize nutrients at low ratios, as they invest in P rich ribosomes required for fast growth (Arrigo, 2005; Klausmeier et al., 2004b). The deficiency of N over P in upwelled waters provides favorable conditions for these organisms, which could reduce the presence of excess phosphate via non-Redfield utilization. This mechanism of P^* reduction was also used to explain the apparent absence of diazotrophic N_2 fixation in the Humboldt upwelling system (Franz et al. 2012b; Mills and Arrigo 2010). This hypothesis was challenged by the recent discovery of heterotrophic nitrogen fixers in OMZ influenced water masses off Peru and Chile (Bonnet et al., 2013; Fernandez et al., 2015; Loescher et al., 2014), which may play a role in reducing the N deficit particularly of water masses below the oxycline (Loescher et al., 2014).

In this study we analyzed nutrient dynamics and stoichiometries of dissolved and particulate organic matter during an expedition in the eastern tropical South Pacific (ETNA) in order to elucidate the mechanisms responsible for P^* consumption and N:P restoration to Redfield proportions in the surface ocean layer off Peru. By means of high performance liquid chromatography (HPLC), a method to determine phytoplankton pigments, we evaluated how non-Redfield nutrient stoichiometries affect the spatial distribution and community composition of diazotrophic and non-diazotrophic phytoplankton.

Materials and Methods

Samples were collected during research expedition M93 on RV Meteor from February 07th until March 09th 2013 in the frame of the Collaborative Research Centre (SFB) 754: Climate–Biogeochemistry Interactions in the Tropical Ocean. In total, 47 stations were sampled between 12°S and 14°S (Fig. 1 A). In this study, we will focus on the three northern transects of our working area. At each station, samples were collected from 3 to 12 discrete depths with either a CTD mounted on a rosette with 24 bottles (10 L) or a pump-CTD system (Strady et al., 2008).

Nutrient concentrations (NO_3^- , PO_4^{3-} and Si(OH)_4) were determined with a QuAatro autoanalyzer (Seal Analytical) directly onboard following Grasshoff et al. (1999). Seawater samples for particulate organic carbon (POC), nitrogen (PON), phosphorus (POP), chlorophyll *a* (Chl *a*) and HPLC analysis were filtered through pre-combusted (5 h at 450°C) 25 mm Whatman GF/F filters (0.7 μm pore size, pressure <200 mbar). For biogenic silica (BSi) analysis, water samples were filtered through 25 mm cellulose acetate filters (0.65 μm pore size, <200 mbar pressure). Filters for POC, PON, POP, BSi and HPLC analysis were immediately stored frozen (-20°C for POC, PON, POP, BSi; -80°C for HPLC) until later analysis.

POC and PON analyses were performed using an elemental analyzer (Euro EA, EuroVector). Prior to analysis, POC filters were placed in an exsiccator containing fuming HCl for 12 h in order to remove particulate inorganic carbon and then dried for 12 h at 60°C. POP was determined photometrically (Hansen and Koroleff, 1999) after the treatment with Oxisolv® (Merck) in order to oxidize all particulate organic phosphorus to orthophosphate. For DOP analysis, 60 mL of sample was filtered through pre-combusted (450°C, 5 hours) 25 mm Whatman GF/F filters (0.7 μm pore size) and stored frozen (-20°C) in acid cleaned HDPE bottles. Prior to analysis, the filtrate was autoclaved with Oxisolv (Merck) for 30 min. Oxidized organic phosphorus was measured spectrophotometrically as phosphate on a QuAatro autoanalyzer (Seal Analytical; Hansen and Koroleff, 1999). DOP concentrations were then determined as the difference between total dissolved phosphorus and dissolved inorganic phosphate. BSi was converted to dissolved silicate while heating the filters in 0.1 mol L⁻¹ NaOH at 85°C for 2 h 15 mins. The dissolved silicate was then determined spectrophotometrically (Hansen and Koroleff, 1999).

Chl *a* concentrations were determined directly onboard. After overnight extraction with 90% acetone, fluorescence was measured with a Turner Trilogy fluorometer, which was previously calibrated with a standardized solution (*Anacystis nidulans*, Walter CMP). Chl *a* was calculated following Parsons et al. (1984).

While Chl *a* is a proxy of phytoplankton biomass, certain accessory pigments (e.g. chlorophylls, carotenoids) are algae-class specific (Trees et al., 2000). Thus, the relationship between accessory pigments to Chl *a* can be used as a measure for phytoplankton community composition (e.g. Gieskes et al., 1988; Greisberger and Teubner, 2007; Mackey et al., 1998; Wright, 1991). In order to extract phytoplankton pigments for HPLC analysis 90% acetone was added to the filters, which were then homogenized with glass beads and centrifuged for 10 min at 5000 rpm. The supernatants were filtered through 0.2 μ m Teflon filters to remove filter debris and the extracts were immediately stored at -80°C. Extracts were later analyzed for pigments via HPLC (Dionex UltiMate® 3000 LC system equipped with an autosampler, a photodiode array and a fluorescence detector, Thermo Scientific), following Barlow et al. (1997). Pigments were identified through comparison with the retention times and spectral properties of standards (DHI Water & Environment, Denmark). The relative contribution of phytoplankton groups to total Chl *a* was calculated using the CHEMTAX matrix factorization software (Mackey et al., 1996). We used an initial ratio matrix that was based on ratios developed by DiTullio et al. (2005) and Mackey et al. (1996) for the equatorial Pacific and Peruvian upwelling region. Slight modifications were made in order to account for the presence of the pigment aphanizophyll (Apha), which can only be found in cyanobacteria (Hertzberg et al., 1971; Jeffrey et al., 2011) and is regarded a potential marker pigment for diazotrophs in fresh water systems (Louda et al., 2015). Since the ratio of Apha to Chl *a* in marine cyanobacteria is not given in the literature, an approximation based on cultural experiments was used (Schluter et al., 2004). Divinyl-chlorophyll *a* (Div *a*) concentrations were directly used as an index for *Prochlorococcus* abundances. Hence, zeaxanthin (Zeax) attributed to *Prochlorococcus* had to be accounted for, since it needed to be excluded from the CHEMTAX calculations. For that, we first calculated the contribution of each algae class to Zeax and subtracted the *Prochlorococcus* Zeax from the initial Zeax concentrations. We further divided our data set into different bins for CHEMTAX calculations in order to account for different

algae class compositions between surface/chlorophyll maximum, deep chlorophyll maximum and mesopelagic zone (see Table 1 for output matrices).

Table 1: Pigment to chlorophyll a (Chl a) ratios of different algae classes calculated by CHEMTAX. Samples were divided into 3 different bins prior to analysis. Abbreviations: Chl b = chlorophyll b, C3 = chlorophyll c3, C2 = chlorophyll c2, Peri = peridinin, 19-But = 19'-butanoyloxyfucoxanthin, Fuco = fucoxanthin, Neox = neoxanthin, Pras = prasinoxanthin, Vio = violaxanthin, 19-Hex = 19'-hexanoyloxyfucoxanthin, Allox = alloxanthin, DD + DT = diadinoxanthin and diatoxanthin, Zeax = zeaxanthin, Myxo = myxoxanthophyll, Apha = aphanizophyll.

Surface / Chl max bin	Chl a	Chl b	C3	C2	Peri	19-But	Fuco	Neox	Pras	Vio	19-Hex	Allox	DD + DT	Lutein	Zeax	Myxo	Apha
Diatoms	0.50	0	0	0.16	0	0	0.30	0	0	0	0	0	0.04	0	0	0	0
Dinoflagellates	0.47	0	0	0.15	0.28	0	0	0	0	0	0	0	0.09	0	0	0	0
Prymnesiophytes	0.36	0	0.13	0.08	0	0	0.13	0	0	0	0.22	0	0.07	0	0	0	0
Chrysophytes	0.26	0	0.09	0.05	0	0.29	0.03	0	0	0	0.03	0	0.25	0	0	0	0
Chlorophytes	0.48	0.10	0	0	0	0	0	0	0	0.05	0	0	0	0.34	0.03	0	0
Cryptophytes	0.78	0	0	0.09	0	0	0	0	0	0	0	0.13	0	0	0	0	0
Prasinophytes	0.41	0.39	0	0	0	0	0	0.04	0.10	0.04	0	0	0	0	0.01	0	0
Synechococcus	0.71	0	0	0	0	0	0	0	0	0	0	0	0	0	0.29	0	0
Colonial cyanobacteria	0.89	0	0	0	0	0	0	0	0	0	0	0	0	0	0.09	0.02	0
Diazotrophic cyanobacteria	0.75	0	0	0	0	0	0	0	0	0	0	0	0	0	0.01	0.13	0.11
2nd Chl max bin	Chl a	Chl b	C3	C2	Peri	19-But	Fuco	Neox	Pras	Vio	19-Hex	Allox	DD + DT	Lutein	Zeax	Myxo	Apha
Diatoms	0.50	0	0	0.16	0	0	0.30	0	0	0	0	0	0.04	0	0	0	0
Dinoflagellates	0.47	0	0	0.15	0.28	0	0	0	0	0	0	0	0.09	0	0	0	0
Prymnesiophytes	0.30	0	0.07	0.07	0	0	0.11	0	0	0	0.39	0	0.06	0	0	0	0
Chrysophytes	0.26	0	0.09	0.05	0	0.29	0.03	0	0	0	0.03	0	0.25	0	0	0	0
Chlorophytes	0.48	0.10	0	0	0	0	0	0	0	0.05	0	0	0	0.34	0.03	0	0
Cryptophytes	0.78	0	0	0.09	0	0	0	0	0	0	0	0.13	0	0	0	0	0
Prasinophytes	0.41	0.39	0	0	0	0	0	0.04	0.10	0.04	0	0	0	0	0.01	0	0
Synechococcus	0.65	0	0	0	0	0	0	0	0	0	0	0	0	0	0.35	0	0
Colonial cyanobacteria	0.89	0	0	0	0	0	0	0	0	0	0	0	0	0	0.09	0.02	0
Diazotrophic cyanobacteria	0.82	0	0	0	0	0	0	0	0	0	0	0	0	0	0.01	0.05	0.12
Mesopelagic bin	Chl a	Chl b	C3	C2	Peri	19-But	Fuco	Neox	Pras	Vio	19-Hex	Allox	DD + DT	Lutein	Zeax	Myxo	Apha
Diatoms	0.52	0	0	0.12	0	0	0.31	0	0	0	0	0	0.05	0	0	0	0
Dinophytes	0.47	0	0	0.15	0.28	0	0	0	0	0	0	0	0.10	0	0	0	0
Prymnesiophytes	0.33	0	0.08	0.07	0	0	0.12	0	0	0	0.33	0	0.06	0	0	0	0
Chrysophytes	0.26	0	0.09	0.05	0	0.29	0.03	0	0	0	0.03	0	0.25	0	0	0	0
Chlorophytes	0.48	0.10	0	0	0	0	0	0	0	0.05	0	0	0	0.34	0.03	0	0
Cryptophytes	0.78	0	0	0.09	0	0	0	0	0	0	0	0.13	0	0	0	0	0
Prasinophytes	0.41	0.39	0	0	0	0	0	0.04	0.10	0.04	0	0	0	0	0.01	0	0
Synechococcus	0.65	0	0	0	0	0	0	0	0	0	0	0	0	0	0.35	0	0
Colonial cyanobacteria	0.89	0	0	0	0	0	0	0	0	0	0	0	0	0	0.09	0.02	0
Diazotrophic cyanobacteria	0.68	0	0	0	0	0	0	0	0	0	0	0	0	0	0.01	0.02	0.29

Results

Hydrographical setting

In February/March 2012, sampling began close to the shore and progressed perpendicular to the coast, thereby crossing the continental shelf (width ~60 km), the shelf break at approximately 77.5°W and the Peru/Chile undercurrent over the continental slope (Fig. 1 B). Coastal upwelling of water from 50 to 100 m water depth occurred between 12° and 14°S, with near surface temperatures of around 17°C on the shelf and around 23°C further offshore (Fig. 2). Mean oxygen (O_2) concentrations at the surface were around $240 \mu\text{mol kg}^{-1}$ and decreased rapidly with depth. O_2 values $<1 \mu\text{mol kg}^{-1}$ were already observed at around 30 m depth above the shelf, while the oxycline deepened further offshore and anoxic waters were encountered at depth around 80 m (Thomsen et al., 2015).

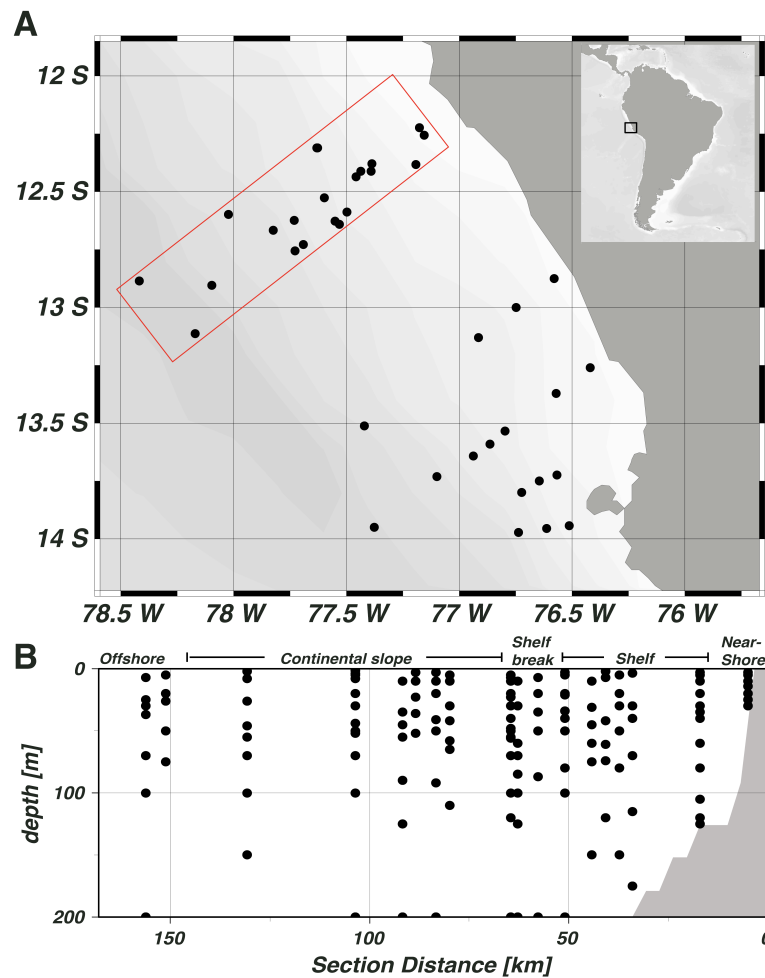


Figure 1: Map of the study area. (A) Sampled stations during research cruise M93 are depicted by black dots. Our analysis focused on the upper three transects of the study area (red box) (B) Horizontal zonation of the study area and vertical sampling distribution, which is consistent throughout all figures.

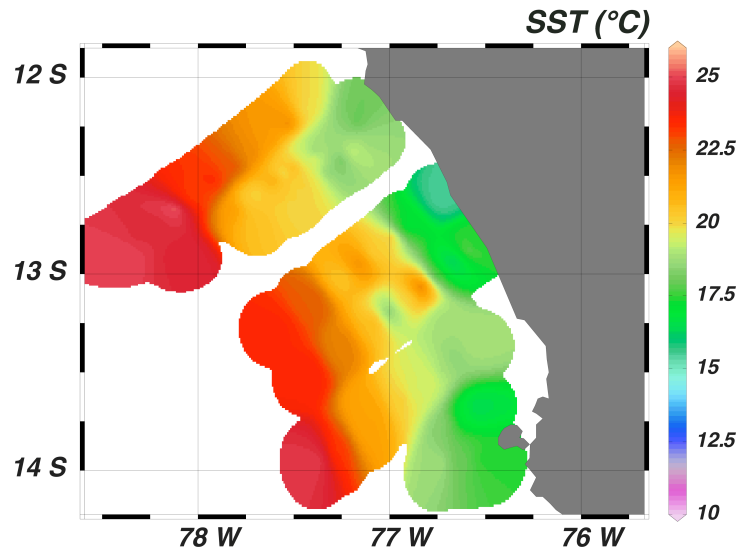


Figure 2: Measured sea surface temperature (SST) in °C in the eastern tropical South Pacific.

Distribution of dissolved inorganic and organic nutrients

Upwelled water masses featured high concentrations of nitrate, phosphate and silicate of around $20 \mu\text{mol L}^{-1}$, $2.5 \mu\text{mol L}^{-1}$ and $15 \mu\text{mol L}^{-1}$, respectively (Fig. 3 A, B, C). Nitrate concentrations were low near the shelf sediment ($0\text{--}3 \mu\text{mol L}^{-1}$), at stations closest to the shore ($0\text{--}1 \mu\text{mol L}^{-1}$) and in surface waters ($0\text{--}1 \mu\text{mol L}^{-1}$). Highest phosphate and silicate concentrations were observed right above the shelf ($3.4 \mu\text{mol L}^{-1}$ and $30 \mu\text{mol L}^{-1}$, respectively) and decreased towards the surface and as waters were transported away from shore. Here, minimum concentrations of $0.3\text{--}0.6 \mu\text{mol L}^{-1}$ for phosphate and $0\text{--}1 \mu\text{mol L}^{-1}$ for silicate were measured. Throughout the study area, N:P ratios never reached Redfield proportions (Fig. 3 D). Maximum values of 12:1 were observed right below the surface layer between 20 to 50 m, while lower values between 2–5:1 were measured in the upper 20 m of the water column. Extremely low values between 0–2 coincided either with the complete absence of nitrate (i.e. above the sediment) or both nitrate and phosphate (i.e. in the surface layer at offshore stations). A surplus of phosphate over nitrate was measured in the whole water column, corresponding to the observations of low N:P values in the study area (Fig. 3 E). Maximum P^* concentrations were measured at near shore stations above the shelf sediment ($3 \mu\text{mol L}^{-1}$), while lowest concentrations of $0.5 \mu\text{mol L}^{-1}$ were found in the upper 40 m of the water column. DOP concentrations in the study area were elevated in the surface layer (Fig. 3 F). Particularly above the shelf, maximum concentrations of up to $0.6 \mu\text{mol L}^{-1}$ were measured down to 50 m water depth. Accompanied

by a shoaling of the nutricline, DOP values decreased to 0–0.2 $\mu\text{mol L}^{-1}$ as water masses were transported offshore.

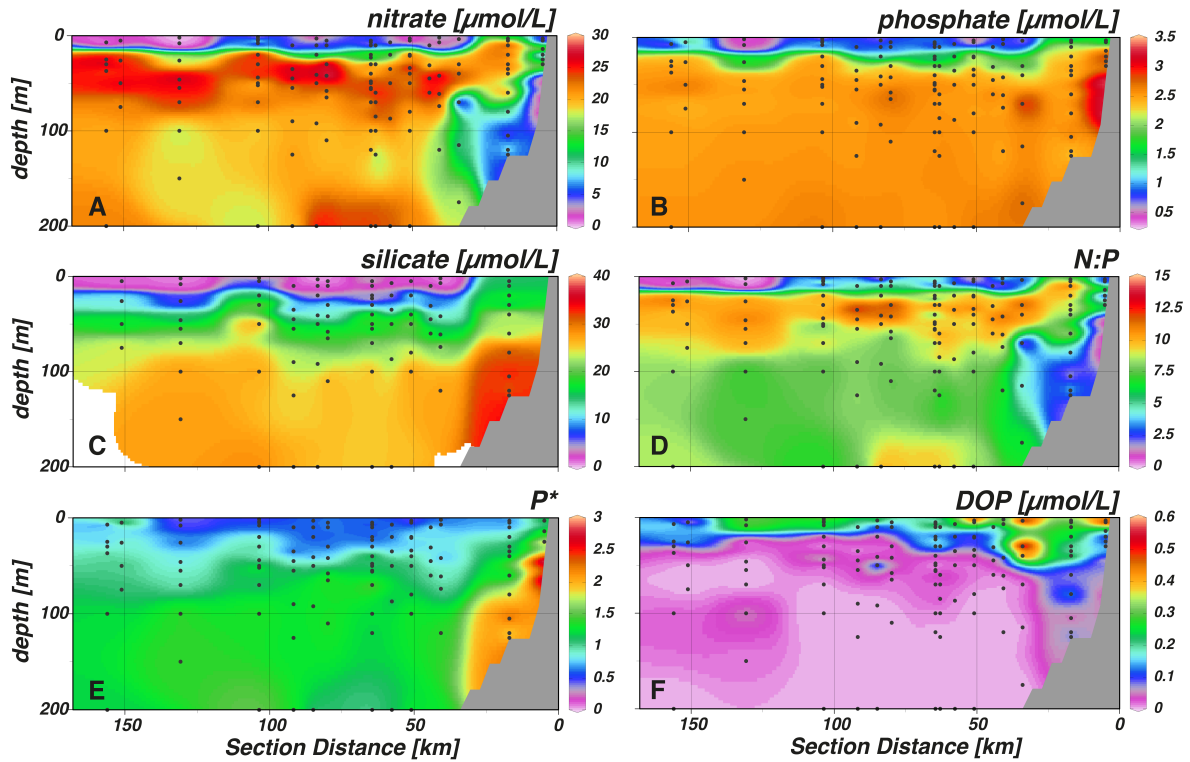


Figure 3: Cross-shelf transects with interpolated concentrations of (A) nitrate, (B) phosphate, (C) silicate, (D) nitrate to phosphate ratio (N:P), (E) excess phosphate (P^*) and (F) dissolved organic phosphorus (DOP).

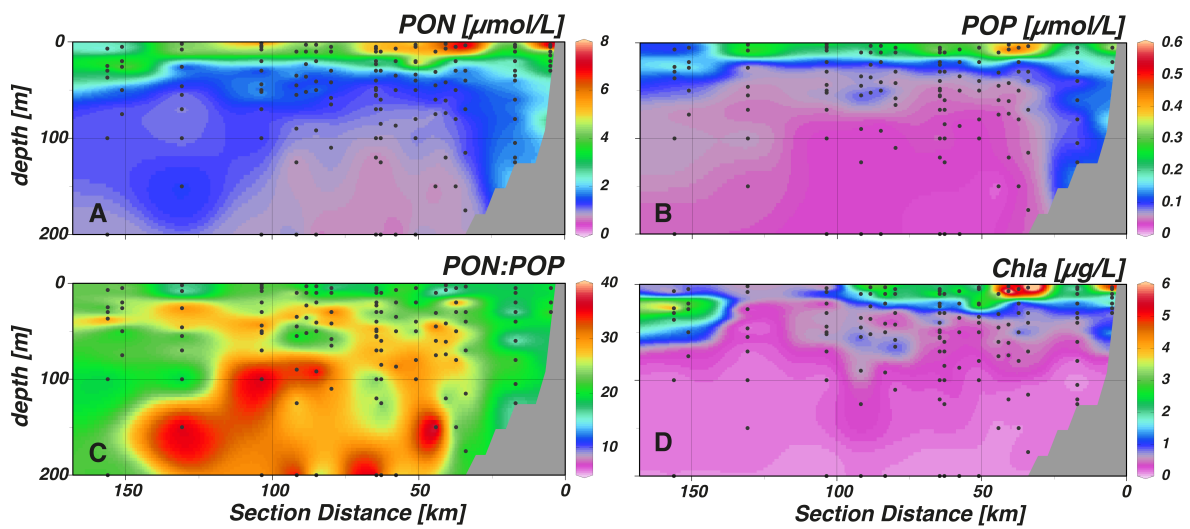


Figure 4: Spatial distribution of (A) particulate organic nitrogen (PON), (B) particulate organic phosphorus (POP), (C) PON:POP and (D) chlorophyll a (Chl a) in our study area.

Particulate organic matter dynamics

Elevated concentrations of PON ($4\text{--}8\ \mu\text{mol L}^{-1}$) were measured in the upper 20–30 m (Fig. 4 A), with maximum values ($\sim 8\ \mu\text{mol L}^{-1}$) observed close to the surface at near shore and shelf stations. Similar to PON, high POP concentrations ($0.3\text{--}0.6\ \mu\text{mol L}^{-1}$) were observed in the upper water column (Fig. 4 B). Despite very low inorganic N:P ratios in the whole study area, PON:POP ratios above Redfield proportions ($\sim 24:1$) prevailed (Fig. 4 C). Only at few stations values of 16:1 or slightly lower were encountered. In general, PON:POP ratios of 16–20:1 were observed at near shore and shelf stations throughout the water column and between 0–20 m depth as water masses were transported offshore. At stations over the continental slope (50–100 km distance to shore) we observed high PON:POP values of $\sim 40:1$ at depths between 40–200 m.

Phytoplankton biomass and composition

Chlorophyll *a* concentrations reached highest values ($\sim 6\ \mu\text{g L}^{-1}$) in the upper 20 m of the near shore and shelf stations ($<50\text{m}$ distance to shore; Fig. 4 D), associated with a community dominated by diatoms (Fig. 5 A). These findings are in agreement with the distribution of biogenic silica (Fig. S1), a mineral synthesized by diatoms and therefore a good indicator for the abundance of this algae class. In addition to diatoms, cryptophytes were present at the near shore stations and high concentrations of prymnesiophytes and prasinophytes were found above the shelf (Fig. 5 B–D). Elevated Chl *a* concentrations ($\sim 3\ \mu\text{mol L}^{-1}$) were also observed in the upper 20–30 m over the continental slope. In terms of Chl *a* biomass, diatoms were again the dominant phytoplankton group, but also prymnesiophytes, prasinophytes, chrysophytes (Fig. 5 E) and the cyanobacteria *Synechococcus* (Fig. 6 A) reached their highest abundances in these areas. At stations further offshore, nutrients were depleted in the surface and prymnesiophytes and *Synechococcus* dominated the algae community. At the same stations, a deep-chlorophyll maximum was observed between 30–50 m depth and diatoms and cryptophytes were highly abundant. Associated to this offshore deep chlorophyll maximum was also the highest abundance of the cyanobacterium *Prochlorococcus* in the study area (Fig. 6 B). This algae group was generally observed at subsurface low-oxygen waters between 30–80 m depth. Abundances of other phytoplankton classes such as dinoflagellates and chlorophytes were negligible in our study area and are therefore not shown.

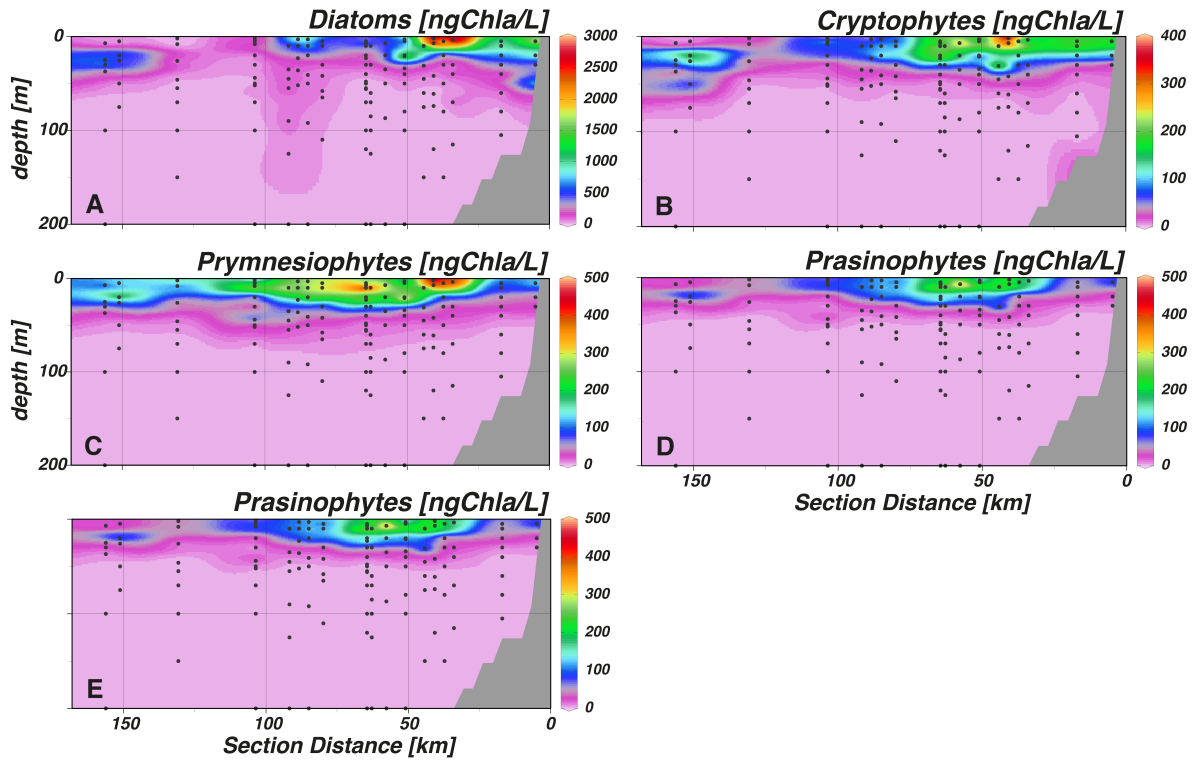


Figure 5: Cross-shelf distribution of phytoplankton classes in the study area. (A) diatoms, (B) cryptophytes, (C) prymnesiophytes, (D) prasinophytes, (E) chrysophytes.

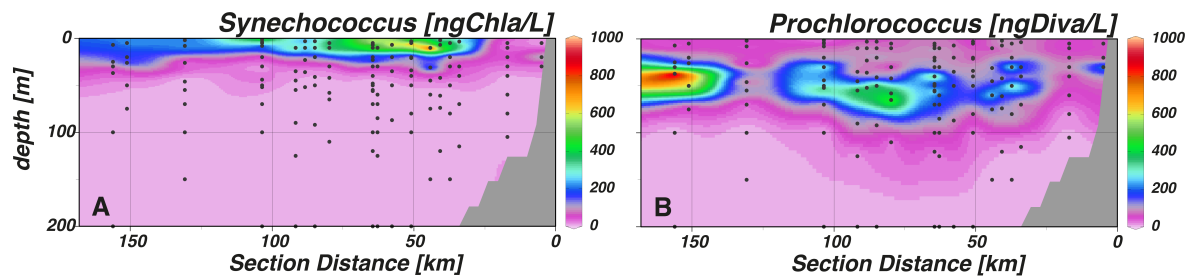


Figure 6: Cross-shelf transects showing the distribution of the cyanobacteria (A) *Synechococcus* and (B) *Prochlorococcus*.

Abundance of diazotrophic cyanobacteria

Aside from pigments indicative for the abundance of *Prochlorococcus* and *Synechococcus*, evidence for the occurrence of other – possibly diazotrophic – cyanobacteria was found in the study area. Colonial cyanobacteria, distinguished by their marker pigment myxoxanthophyll, were present in the surface layer of the near shore and shelf stations (Fig. 7 A). Lower abundances were observed close to the surface at outer shelf stations and in the deep chlorophyll maximum at the offshore stations. Diazotrophic cyanobacteria, represented by the accessory pigments aphanizophyll, were most abundant in the upper 40 m at the near shore stations but also showed higher abundances in surface waters of the shelf and above the continental slope (Fig. 7 B).

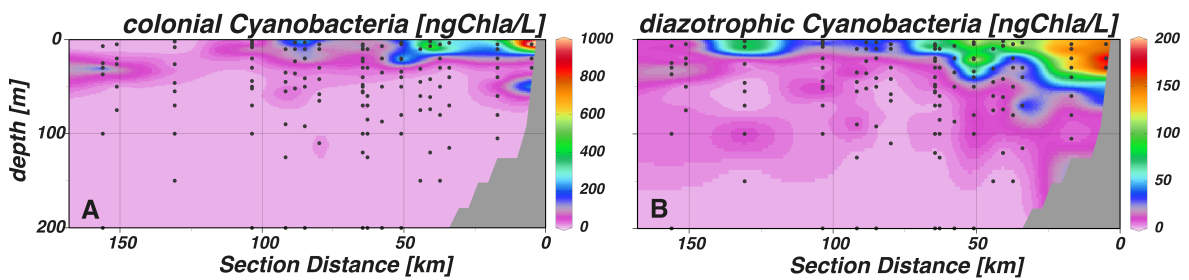


Figure 7: Spatial distribution of (A) colonial and (B) diazotrophic cyanobacteria in our study area.

Discussion

Phytoplankton succession and particulate organic matter stoichiometry

Upwelling and the associated supply of nutrients to the surface ocean fuelled high primary production in the ETSP for the duration of our cruise in austral summer 2012. The near shore and shelf Chl *a* maximum was dominated by diatoms, which is characteristic for the Peruvian upwelling system (Chavez et al., 1996; Franz et al., 2012b; Pennington et al., 2006). Due to their high growth rate (Sarhou et al., 2005) and nutrient storage capacity (Raven, 1997) diatoms outcompete other algae groups when nutrients are abundant. Over the continental slope, mixtures of different phytoplankton groups were present, consisting of diatoms, prymnesiophytes, prasinophytes and chrysophytes. As waters were transported offshore, silicate was depleted in the surface ocean and the phytoplankton assemblage changed from a diatom dominated community to an assemblage of non-siliceous phytoplankton groups, where prymnesiophytes and the cyanobacterium *Synechococcus* prevailed. In subsurface waters, elevated PON:POP ratios (~30:1) coincided with a high abundance of *Prochlorococcus*, which are known to exhibit higher than Redfield N:P ratios due to their slow growth rates (Bertilsson et al., 2003; Biller et al., 2015). Presence of this picophytoplankton group extended below the oxycline and prevailed throughout the study area. Low light adapted *Prochlorococcus* strains have been identified in different OMZs (Beman and Carolan, 2013; Goericke et al., 2000) including the ETSP (Lavin et al., 2010; Ras et al., 2008). Due to their small size and unique pigment composition they are highly adapted to low light levels (Moore et al., 1998) and thus can make use of the higher nutrient load available at depth.

A distinct succession of phytoplankton species from onshore to offshore has previously been recognized in the Peruvian upwelling system (Franz et al., 2012b). This study reported that very low inorganic N:P ratios in the water column directly translated into low cellular N:P ratios in the microorganisms. The authors argued that there is a linear relationship between available and cellular N:P ratios and that low nutrient stoichiometries in the water column selected for certain algae groups with lower cellular N:P quotas, supporting the hypothesis of Arrigo (2005), Klausmeier et al. (2004b) and Sterner and Elser (2002). Non-Redfield nutrient utilization by these organisms consumed

the excess P in the water column and thereby restored the stoichiometry of inorganic nutrients back to Redfield proportions as waters were transported offshore.

During our study, a pronounced surplus of P over N was measured in waters that were transported to the surface by upwelling, a consequence of N loss processes within the Peruvian OMZ (Dalsgaard et al., 2012; Kalvelage et al., 2013) and high concentrations of P, which were released from the sediment under reducing conditions (cf Fig. 3 B; Noffke et al., 2007). However, despite very low inorganic N:P ratios of 2.5–10 at the inner shelf stations and the surface layer further offshore, PON:POP ratios in these areas were close to or even above Redfield proportions, with no indication of non-Redfield nutrient uptake. At the same time, high P^* values declined relatively fast as waters were transported to the surface and away from the coast and N:P draw down ratios were low, which conflicts with the apparent absence of non-Redfield production.

The observed deviation of PON:POP ratios from inorganic nutrient stoichiometries, accompanied by decreasing P^* values, can be explained by different mechanisms: (1) non-Redfield nutrient assimilation reduced P^* , while the surplus of phosphorus in the biomass was released as DOP, (2) the particulate organic matter pool had a large detrital component which was enriched in N relative to P, resulting in higher PON:POP ratios and/or (3) nutrient assimilation was according to Redfield and excess phosphate in the water column was reduced by N_2 fixation.

Non-Redfield nutrient assimilation

Theoretical and experimental approaches showed that the availability and stoichiometry of nutrients in seawater can induce differences in cellular composition of phytoplankton (Franz et al., 2012a; Geider and La Roche, 2002; Mills and Arrigo, 2010; Moore et al., 2008; 2013). During our study, low inorganic nutrient supply ratios, decreasing P^* values and low concentrations of both nitrate and phosphate in the surface ocean suggest that nutrients were assimilated in non-Redfield proportions. We suggest that the surplus of phosphorus in the biomass was transferred from the particulate into the dissolved organic phosphorus pool, confirmed by the presence of elevated DOP concentrations in the surface close to shore. ‘Luxury’ P uptake and subsequent DOP release under P replete conditions has been previously observed in mesocosm experiments (Meyer et al., 2016; Ruttenberg and Dyhrman, 2012).

Mackey et al. (2012) argued that this channeling of P into DOP might be an important part of the P cycle in upwelling regions. Instead of P being transported out of the euphotic zone by export production, it is retained and remains available for phytoplankton.

Non-Redfield production might have further been masked by remineralization of phosphorus from particulate organic matter (POM). Large parts of POM in our study area do not appear to be freshly produced, as the surface POC:Chl *a* ratio is considerably higher ($>100:1$; Fig. S2) than previously reported for the ETSP upwelling regime ($\sim 50:1$; Chavez et al., 1996) or as described for diatom-dominated communities ($15:1$ – $75:1$; e.g. Sathyendranath et al., 2009; Lorenzoni et al., 2015). This implies that the POM we encountered had a large detrital component. As organic phosphorus is known to be remineralized more rapidly than carbon and nitrogen (Burkhardt et al., 2014; Kolowitz et al., 2001; Loh and Bauer, 2000), elevated PON:POP ratios could be the result of selective degradation of POP.

Co-occurrence of P* consumption and diazotroph abundance

It has previously been shown that certain phytoplankton communities and/or species do not adjust their internal stoichiometries to match low nutrient ratios in the surrounding medium (Hall et al., 2005; Meyer et al., 2016). Other studies suggest that phytoplankton nutrient assimilation follows their optimal uptake ratio under nutrient replete conditions (Klausmeier et al., 2004a) and is more dependent on the growth rate of individual species and algae communities (Goldman et al., 1979; Hillebrand et al., 2013) than on the initial nutrient supply ratio. Thus, PON:POP ratios close to or higher than Redfield proportions during our research cruise might also be explained by phytoplankton utilizing nutrients in Redfield proportions. As an excess of phosphate over nitrate and/or high concentrations of DOP are thought to create a niche for diazotrophic organisms (Björkman and Karl, 2003; Deutsch et al., 2007; Mahaffey et al., 2014; Sohm and Capone, 2006), P* might have been consumed by N₂ fixers. Indeed, we observed highest abundances of diazotrophs in areas where we measured elevated DOP and P* concentrations. Especially colonial cyanobacteria, represented by the marker pigment aphanizophyll, were widely present in surface waters of near shore and shelf stations and also occurred at stations further offshore. Aphanizophyll is a pigment that can be found in heterocyst forming diazotrophs like *Aphanizomenon* spp. and *Anabaena* spp., which live in brackish or estuarine

waters (Hertzberg and Liaaen-Jensen, 1971) and is indicative for nitrogen fixing cyanobacteria in fresh water systems (Donald et al., 2013; Louda et al., 2015 and references therein). In the oceanic environment, reports of the detection of aphanizophyll are extremely scarce. It has, however, been detected after the decline of a diatom bloom in mesocosm experiments off Peru (Hausse et al., 2012) and was also attributed to the existence of N_2 fixing cyanobacteria. At the near shore and shelf stations, we also detected high abundances of colonial cyanobacteria, indicated by the marker pigment myxoxanthophyll. This carotenoid can be found in marine N_2 fixing cyanobacteria (Carpenter et al., 1993; Franz et al., 2012b; Schluter et al., 2004), but is regarded a general marker for colonial cyanobacteria in fresh water environments, where it can also be found in non-diazotrophic cyanobacteria (Louda et al., 2015).

Although N_2 fixation was already suggested to take place in the vicinity of upwelling regions (Karl and Letelier, 2008) and in close spatial coupling to denitrification (Deutsch et al., 2007), the existence of diazotrophic cyanobacteria in nutrient replete surface waters of upwelling regions is counterintuitive when considering the classical paradigm that high concentrations of reactive nitrogen compounds inhibit diazotrophy (Tyrrell, 1999; Ward et al., 2013). However, there is growing evidence that N_2 fixation occurs under N-rich conditions (Dekaezemacker and Bonnet, 2011; Knapp, 2012; Knapp et al., 2012; Meyer et al., 2016) and even within upwelling regions of the Benguela and equatorial Atlantic (Sohm et al., 2011; Subramaniam et al., 2013). In the Peruvian upwelling region, the high supply of iron and phosphate from the sediment might stimulate growth of diazotrophs, thereby allowing them to compete with other phytoplankton groups. Further offshore, diminished iron supply limits algae growth (Hutchins et al., 2002), consequently also restricting the growth of diazotrophs.

Conclusion

During a research expedition to the Humboldt Current system in austral summer 2012, we investigated the phytoplankton community composition and response to low N:P ratios in water masses influenced by the Peruvian OMZ. Our study confirmed that a variety of phytoplankton species coexist in this dynamic ecosystem. A considerable portion of excess phosphorus in the surface was reduced as water masses were transported away from the shore. The data presented here suggest that there are several mechanisms responsible for P* removal in the ETSP. While non-Redfield nutrient utilization as one of the mechanisms could not be ruled out, evidence for the presence of diazotrophic organisms in the area was also detected. The recent discovery of novel *Trichodesmium* phylotypes (Turk-Kubo et al., 2014) and other unknown diazotrophs (Sohm et al., 2011) in upwelling regions of the ETSP and Benguela and our observations of previously undetected cyanobacterial marker pigments in the Peruvian upwelling region add to the growing body of evidence that there are still unknown communities of autotrophic and heterotrophic diazotrophs (Bonnet et al., 2013; Loescher et al., 2014) that exist in environments previously not considered relevant for nitrogen fixation. Uncertainties concerning the identity and activity of diazotrophs in the Peruvian upwelling regions need to be addressed in future studies in order to elucidate sensitivities and constraints of N₂ fixation in the ocean.

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All data will be uploaded to www.pangaea.de upon publication.

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Appendix

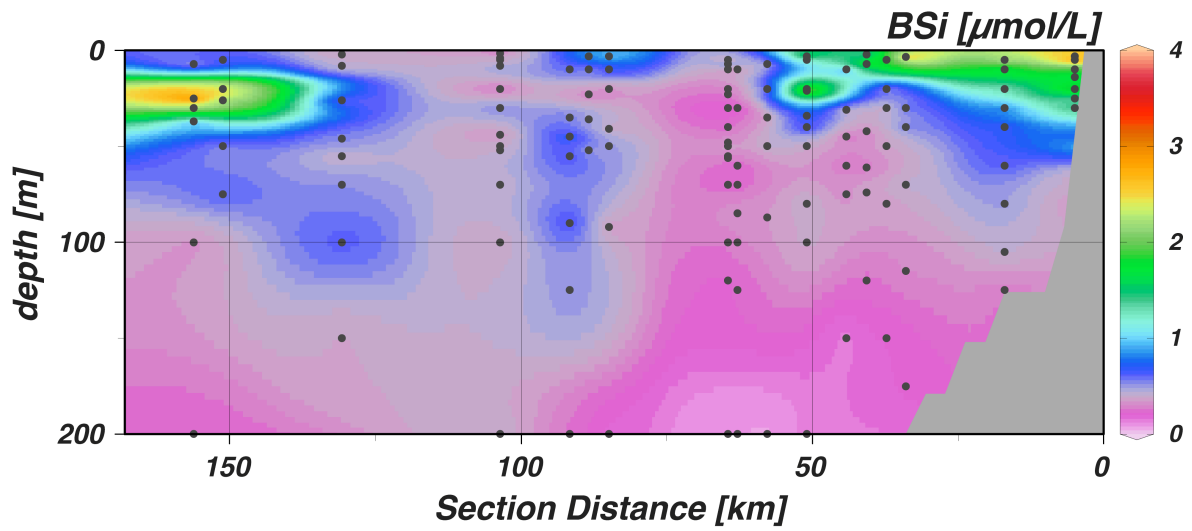


Figure S1: Cross-shelf transect showing the interpolated concentration of biogenic silica (BSi).

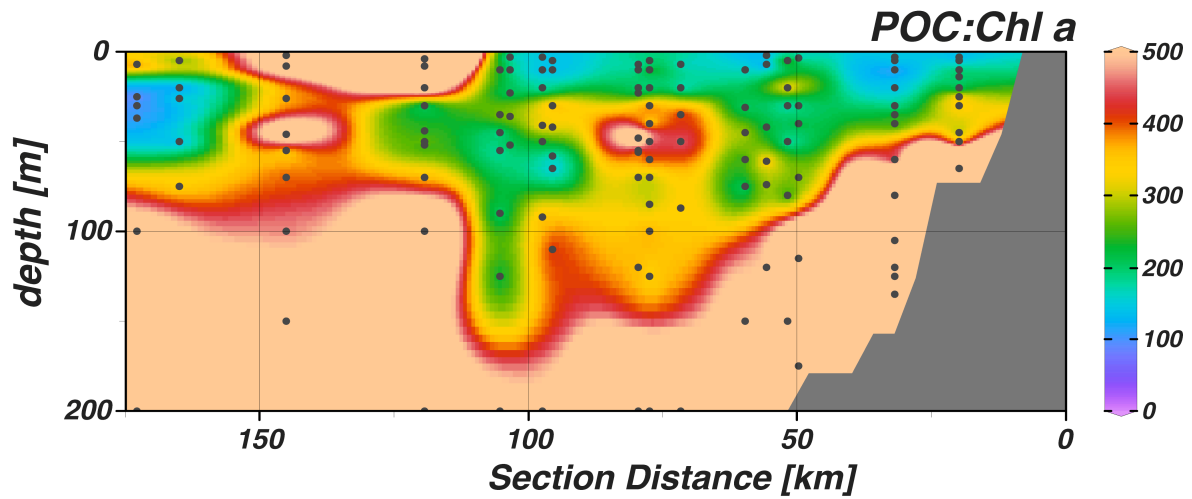


Figure S2: Particulate organic carbon (POC in $\mu\text{g/L}$) to chlorophyll a (Chl a in $\mu\text{g/L}$) ratios in the upper 200 m of the eastern tropical South Pacific.

Dissolved phosphorus compounds enhance N₂ fixation rates in the eastern tropical North Atlantic

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to be submitted

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Abstract

In the coastal upwelling system of the eastern tropical North Atlantic (ETNA), dissolved organic phosphorus (DOP) production and its release by phytoplankton is increasingly recognized as an important supply mechanism for phosphorus (P) to the oligotrophic open ocean. Photoautotrophs and dinitrogen (N_2) fixing organisms (diazotrophs) are thought to be able to consume DOP, thus alleviating P stress in areas with extremely low dissolved inorganic phosphate (DIP) concentrations. In the present study, nutrient addition bioassay experiments were conducted to investigate the bioavailability of different organic and inorganic phosphorus components to the phytoplankton community in the ETNA. We specifically examined how DIP and DOP amendments affected N_2 fixation in that area. Our observations showed that phytoplankton growth was primarily limited by nitrogen. Moreover, DIP addition resulted in a significant increase in N_2 fixation rates in almost all experiments relative to control treatments, suggesting that diazotrophs were limited by P availability in our study. At very oligotrophic sampling stations, all P compounds stimulated N_2 fixation rates compared to the control. This demonstrated the capability of the diazotrophic community to utilize various DOP compounds, especially under P limiting conditions. Our findings provide a mechanism explaining how high N_2 fixation rates can be sustained under extremely low phosphate concentrations in the oligotrophic North Atlantic.

Introduction

Phosphorus (P) is an essential element for life and one of the major nutrients supporting ocean primary productivity. In the cell, P is an important structural component (RNA, DNA, cell wall) and a central molecule in the energy transport system (adenosine triphosphate). The most bioavailable form of P in the ocean is dissolved inorganic phosphate (DIP), whereas dissolved organic phosphorus (DOP) is not as readily available for primary producers (Björkman and Karl, 2003).

Unlike nitrogen (N), iron (Fe) and silica (Si), which are considered the most important nutrients limiting phytoplankton growth in the world's oceans, P is often thought to only limit primary production on geological time scales (e.g. Redfield, 1958; Tyrrell, 1999). In certain regions, however, surface phosphate concentrations are very low. Especially in the subtropical and tropical North Atlantic, a deficiency of inorganic P over inorganic N (with reference to the canonical Redfield ratio of $N:P = 16:1$; Redfield, 1958) is often present (Ammerman et al., 2003; Capone et al., 2005; Moore et al., 2008) and surface DIP concentrations can limit or co-limit primary production (Mills et al., 2004; Moore et al., 2013; Wu et al., 2000). Low DIP concentrations in the North Atlantic have mainly been attributed to the high abundance of dinitrogen (N_2) fixing organisms (diazotrophs) (Capone et al., 2005; Mahaffey, 2003; Mather et al., 2008) which can grow independently of bioavailable nitrogen forms (i.e. ammonium, nitrate and nitrite). The high supply of iron via aeolian dust deposition in this region (Jickells et al., 2005) creates favorable conditions for diazotrophs, which draw down phosphate in the water column, thereby increasing N:P ratios.

In oligotrophic realms, where DIP concentrations are chronically low, DOP can be the main P pool, comprising more than 75% of total dissolved P (Karl and Björkman, 2002; Mather et al., 2008; Sohm and Capone, 2010). In the subtropical North Atlantic, a major fraction of DOP is produced on the shelf (Reynolds et al., 2014), where upwelling of nutrient replete water masses supports primary production and an excess of phosphate over nitrate in the water column promotes the release of DOP from the particulate organic P pool (Davis et al., 2014; Mackey et al., 2012; Meyer et al., 2016). Lateral transport from the shelf region subsequently supplies DOP to the oligotrophic subtropics (Reynolds et al., 2014). Although DOP is not yet fully characterized, it was found to consist of two major component classes: phosphonates and P esters

(e.g. Clark et al., 1998; Karl and Yanagi, 1997). P esters are the dominant DOP form in the surface ocean and seem to be more readily available for microorganisms, whereas phosphonates are thought to cycle more slowly (Kolowitz et al., 2001; Young and Ingall, 2010). As DOP compounds cannot cross the cell membrane, microbes need to hydrolyze them outside the cell. Extracellular enzymes bound to the cell-surface, such as alkaline phosphatase and C-P lyase, catalyze this reaction, which leads to the release of DIP from organic substrates (Cembella et al. 1984).

While phytoplankton have a strong metabolic preference for inorganic P, DOP has been increasingly recognized as a substrate supporting primary production in oligotrophic regions (Lomas et al., 2010; McLaughlin et al., 2013). In the tropical and subtropical North Atlantic, where diazotrophs were shown to be limited or co-limited by the availability of DIP (Mills et al., 2004; Moore et al., 2009; Sohm et al., 2008), N₂ fixers are hypothesized to access DOP compounds in order to satisfy their P requirements (Dyhrman et al., 2006; Sohm and Capone, 2006). Linking to this, results from a recent mesocosm study hint towards a possible connection between phosphate limitation, DOP utilization and N₂ fixation in the eastern tropical North Atlantic (Meyer et al. 2016).

In order to test whether DOP is available to phytoplankton and diazotrophs, nutrient addition bioassays were conducted during a research cruise in the eastern tropical North Atlantic. At six stations, surface seawater was incubated with either DIP or one of two different DOP sources, both of which are largely abundant in the natural P pool (Kolowitz et al., 2001). We followed the evolution of chlorophyll *a* (Chl *a*) as an indicator for phytoplankton biomass and carried out N₂ fixation rate measurements to assess whether: (1) diazotrophs in the sampling area are limited by P availability, (2) DOP enhances N₂ fixation and (3) bioavailability differs between DIP and DOP compounds.

Materials and Methods

Experimental set up

In March/April 2014 six bioassay experiments (B1-B6) were carried out during RV Meteor cruise M105 in the eastern tropical North Atlantic (Fig. 1). For each experiment, seawater from 5 m depth was collected from CTD casts and directly filled into 24 4.4 L polycarbonate bottles (Nalgene, Thermo Fisher Scientific, USA), which were acid cleaned (10% HCl) and rinsed three times with sample water before filling. The bottles were placed into on-deck incubators, which were connected to a flow-through seawater system and maintained in situ surface temperatures ($\sim 21\text{--}26^\circ\text{C}$) throughout the experiment. Incubators were shaded with blue lagoon light foil to simulate light levels at 5 m water depth.

Each bioassay ran for three days. Samples for nutrient and chlorophyll analyses were taken before the nutrient addition and after 24, 48 and 72 hours. Duplicate incubation bottles were spiked with either potassium dihydrogen phosphate as dissolved inorganic phosphate (DIP) source, glucose-6-phosphate (G-6-P) as dissolved organic phosphorus (DOP) source or adenosine monophosphate (AMP), which is usually classified as DOP but also comprises nitrogen compounds. This treatment will therefore be referred to as dissolved organic nitrogen (DON)+DOP treatment. Final nutrient concentrations were $2\ \mu\text{mol L}^{-1}$ DIP, $1\ \mu\text{mol L}^{-1}$ DOP and $1\ \mu\text{mol L}^{-1}$ DON +DOP. Control incubations (no nutrients additions) were conducted in parallel.

Nutrient and chlorophyll analyses

Samples (10 mL) for nitrate + nitrite (hereafter referred to as nitrate or NO_3^-) and phosphate (PO_4^{3-}) analyses were immediately stored frozen (-20°C) after sampling and analyzed onshore within 2 months after collection. The analyses were conducted using a QuAAtro auto-analyzer (SEAL Analytical) and followed the method of Hansen and Koroleff (2007). Triplicate nutrient samples were taken to determine the precision of the measurement. Obtained precisions were $0.08\ \mu\text{mol L}^{-1}$ (nitrate), $0.02\ \mu\text{mol L}^{-1}$ (nitrite) and $0.05\ \mu\text{mol L}^{-1}$ (phosphate). P^* was calculated after Deutsch et al. (2007) as: $\text{P}^* = \text{PO}_4^{3-} - \text{NO}_3^- / 16$.

Samples (0.5–1 L) for Chl *a* were gently filtered (200 mbar) onto Whatman GF/F filters (pore size: $0.7\ \mu\text{m}$) and stored in the dark at -20°C until

measurements. For the analysis, 10 mL of 90% acetone was added to the filters. Thereafter, filters were homogenized with glass beads for 5 minutes and centrifuged at 5500 rpm and 0°C for 10 minutes. The supernatant was removed and the fluorescence was measured using a Turner Designs 10-AU fluorometer.

N₂ fixation rates

After 48 hours of incubation, all incubation bottles were amended with ¹⁵N-N₂-enriched seawater, following the protocol by Mohr et al. (2010). In detail, ¹⁵N-N₂-enriched seawater was prepared from water samples collected from the same site as water samples for incubations. The water was filtered through an Isopore polycarbonate filter (pore size: 0.22 µm) and pumped through a degassing membrane (Membrana, Minimodule) connected to a water-jet pump to remove ambient N₂. The degassed water was collected in a gas-tight, acid-cleaned Tedlar® bag and amended with 1 mL of ¹⁵N-N₂ gas (98 atom% ¹⁵N, ISOTECH Lot no.: TV 533, Sigma Aldrich Lot no.: SZ 1423V) for every 100 mL of water sample. After complete dissolution, 100 mL aliquots of this ¹⁵N-N₂ enriched water were added to every incubation bottle without leaving any headspace. The incubations were terminated after 24 hours by filtration onto pre-combusted (450°C, 5 hours) Whatman GF/F filters (pore size: 0.7 µm) under low pressure (<200 mbar). Filters were stored frozen (-20°C) until measurements. For isotope analyses, filters were fumed with HCl (37%) for 24 hours, dried and wrapped into tin cups. Samples were analyzed for particulate organic nitrogen (PON) and isotopic composition according to (Sharp, 1974) using an elemental analyzer (EuroEA) coupled to an isotope ratio mass spectrometer (Thermo Finnigan DeltaPlus XP). N₂ fixation rates were calculated according to Montoya et al. (1996).

Statistical analysis

For each bioassay, a one-way ANOVA was used to compare mean responses between nutrient treatments. Significant differences between means were identified using the Tukey-Kramer test ($\alpha = 0.05$). Due to the small sample size ($n = 2$), the performed statistical tests may not have been powerful enough to detect significant differences between treatments even if the means are very different from each other (see for example Fig. 4, B1, control vs. DOP). A higher number of replicates would have increased the statistical power.

Unfortunately expanding the experiment was not possible due to the limited capacity of the incubators.

The relationship between initial P^* and N_2 fixation response was determined using linear regression analyses (SigmaPlot, Systat).

Results

Initial conditions

Sea surface temperature in the sampling area showed a latitudinal gradient (Fig. 1) with lowest temperatures recorded at the northern stations B1 and B2 ($\sim 21\text{--}23^\circ\text{C}$) and highest temperatures measured at the southern stations B5 and B6 ($\sim 25\text{--}26^\circ\text{C}$). Initial surface nutrient concentrations differed markedly among the six bioassay stations (Fig. 2). Concentrations were highest at station B2, with nitrate and phosphate levels of $4.0\ \mu\text{mol L}^{-1}$ and $0.5\ \mu\text{mol L}^{-1}$, respectively. Very oligotrophic conditions were encountered at the southernmost stations B5 and B6. Here, surface nitrate concentrations were between $0.03\text{--}0.04\ \mu\text{mol L}^{-1}$ and phosphate concentrations were between $0.01\text{--}0.03\ \mu\text{mol L}^{-1}$. At stations B1, B3 and B4 surface nitrate concentrations of $0.5\ \mu\text{mol L}^{-1}$, $0.6\ \mu\text{mol L}^{-1}$ and $1.0\ \mu\text{mol L}^{-1}$, respectively, were detected.

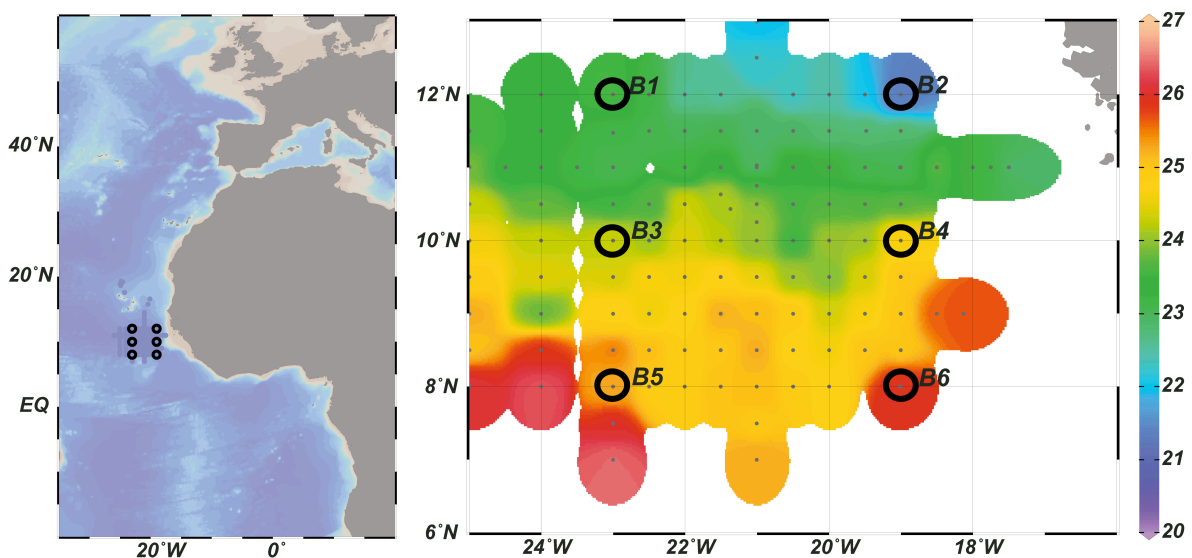


Figure 1: Map of the sampling area in the North Atlantic and location of the six bioassay stations on a map showing sea surface temperature ($^\circ\text{C}$) during the time of our study.

Phosphate levels at these stations were $0.1\ \mu\text{mol L}^{-1}$ (B1) and $\sim 0.2\ \mu\text{mol L}^{-1}$ (B3, B4). P^* values also differed between stations, with highest concentrations of $0.27\ \mu\text{mol L}^{-1}$ at station B2, intermediate values between 0.08 and $0.12\ \mu\text{mol L}^{-1}$ at stations B1, B3 and B4 and low values of $\sim 0.03\ \mu\text{mol L}^{-1}$ at station B5. At station B6, no excess phosphate was detectable in the upper 20 m of the water column. DOP concentrations were below the detection limit in most of the stations, except for B1 and B6, where low concentrations of 0.2

and $0.1 \mu\text{mol L}^{-1}$ DOP were measured (Table 1). In contrast, dissolved organic nitrogen (DON) concentrations were high at all stations, and values were between 3.7 and $6.7 \mu\text{mol L}^{-1}$.

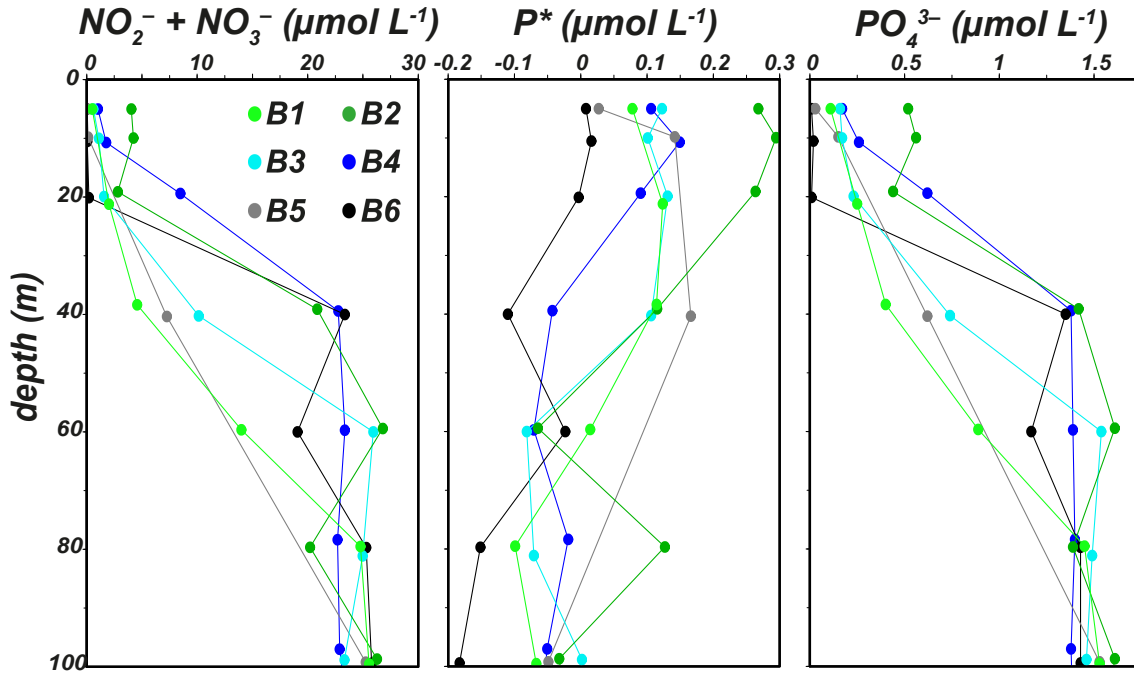


Figure 2: Vertical distribution (0–100 m) of nitrite+nitrate ($\text{NO}_2^- + \text{NO}_3^-$), phosphate (PO_4^{3-}) and excess phosphate ($P^* = \text{PO}_4^{3-} - \text{NO}_3^- / 16$) at the sampling stations. Concentrations are shown in $\mu\text{mol L}^{-1}$.

Table 1: Surface concentrations of DON and DOP (in $\mu\text{mol L}^{-1}$) at the stations sampled.

Station	DON	DOP
B1	5.6	0.2
B2	3.8	0
B3	3.7	0
B4	3.9	0
B5	6.7	0
B6	6.5	0.1

Response of the bulk phytoplankton community

Similar Chl *a* response patterns were observed in all bioassays after the nutrient addition (Fig. 3). In all incubations, DON+DOP stimulated Chl *a* accumulation compared to the control treatments. A significant difference between the control and DON+DOP treatments was already observed after 24 hours in B1, B5 and B6 ($p < 0.05$) and after 48 hours in all other bioassays

amended with DON+DOP. After 72 hours, the Chl *a* increase was 2–6 fold higher in DON+DOP incubations than in the control treatments. No significant increase was observed between control bottles and those amended with DOP in any of the experiments. This indicates that in DON+DOP treatments DON alone was responsible for the strong Chl *a* response observed in the experiments.

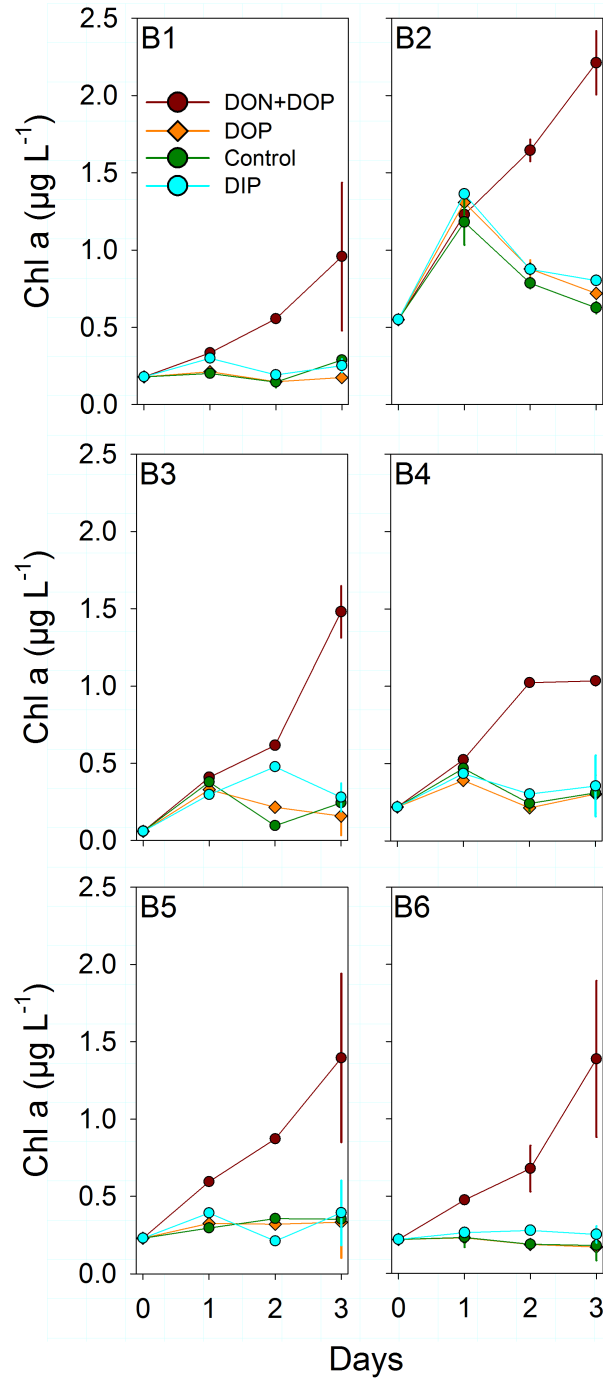


Figure 3: Evolution of chlorophyll *a* ($\mu\text{g L}^{-1}$) in the six bioassay experiments after nutrient addition and in control treatments. Treatment means and standard deviations are displayed.

The addition of DIP caused a slight but significant increase in Chl *a* concentrations in three of the six experiments (B1, B3, B5). This difference between control and DIP treatments was, however, only observed after 24 or 48 hours, respectively.

Nitrogen fixation rates

In all experiments, N₂ fixation was measurable after 72 hours of incubation (Fig. 4). In control treatments, lowest N₂ fixation rates were measured in experiment B2 (~0.2 nmol L⁻¹ h⁻¹), while highest rates of 0.7 nmol L⁻¹ h⁻¹ were observed in B5. In 4 experiments amended with DON+DOP (B1-B4) N₂ fixation rates did not differ from the control treatments or significantly decreased relative to the control. In experiments B5 and B6 N₂ fixation was stimulated by the addition of DON+DOP and maximum N₂ fixation rates of ~1 nmol L⁻¹ h⁻¹ were measured. An increase in N₂ fixation of similar proportions was observed in experiments B5 and B6 amended with DOP. Although not statistically significant, increased N₂ fixation rates were observed in experiments B1 and B4 amended with DOP, while no response was observed in B2 and B3. Amendments with DIP stimulated N₂ fixation rates in all but one experiment. A significant ($p < 0.05$) increase was observed in B4, B5 and B6. Here, the increase was 1.7-2 fold compared to the unamended control. In all treatments, the N₂ fixation response to nutrient addition (determined as $\Delta N_2 \text{ fixation} = N_2 \text{ fixation}_{\text{treatment}} - N_2 \text{ fixation}_{\text{control}}$) correlated significantly with initial P* values, that were determined at the sampling sites (Fig. 5). The strongest correlation was observed for DIP treatments ($r^2 = 0.76$, $p < 0.001$), while a moderate correlation was detected for DOP and DON+DOP treatments (DOP: $r^2 = 0.47$, $p < 0.05$; DON+DOP: $r^2 = 0.50$, $p < 0.05$).

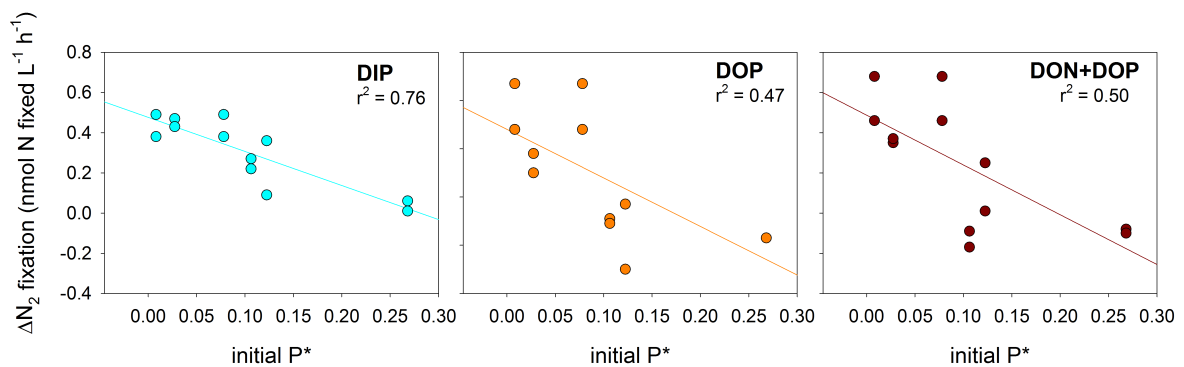


Figure 5: Relationship between the N₂ fixation response, calculated as the difference between N₂ fixation rates of treatment and control, and the surface P* concentration at the stations sampled. Regression analyses were statistically significant ($p < 0.05$) in all treatments. The color code is the same as in the other figures.

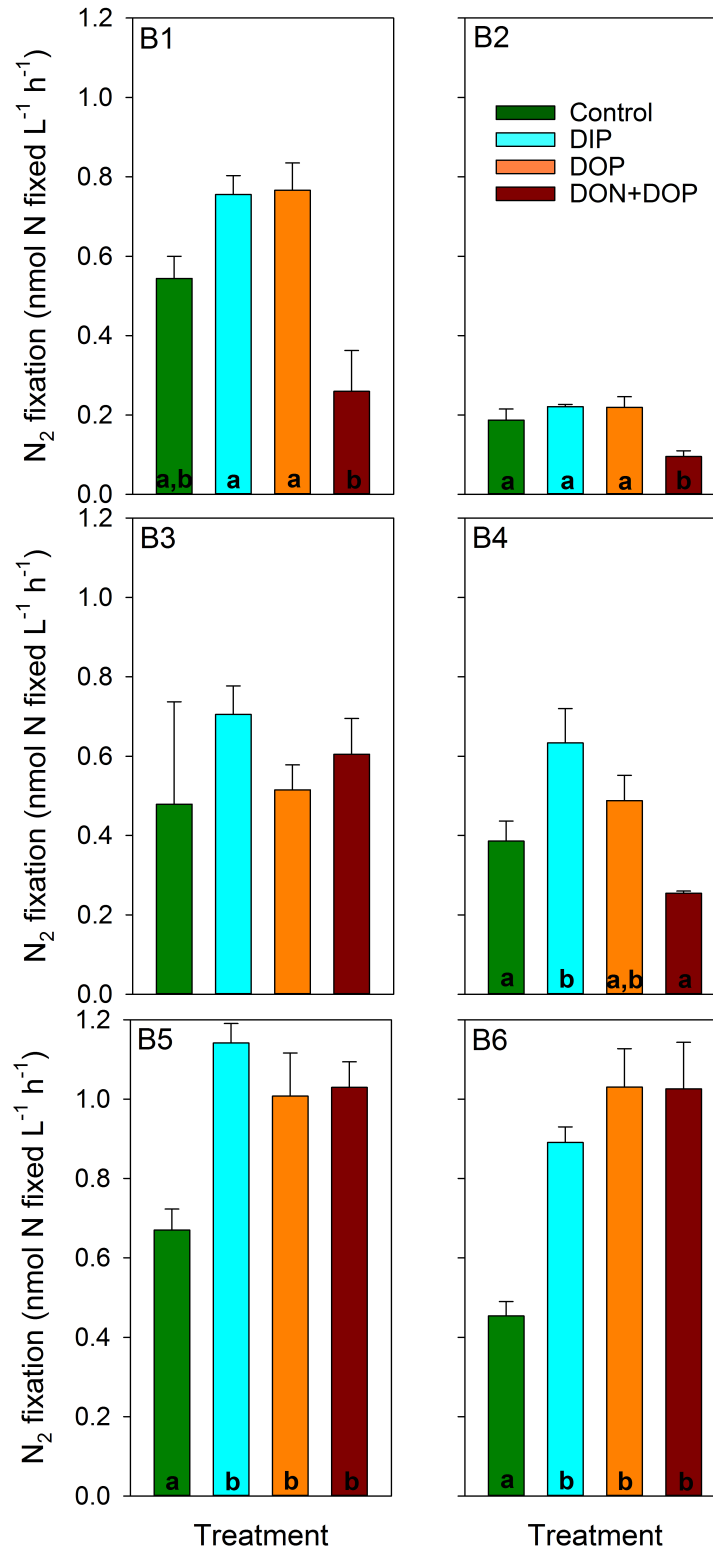


Figure 4: Nitrogen fixation rates (nmol $L^{-1} h^{-1}$) in response to nutrient addition and in control treatments. Rates were determined 72 hours after nutrient addition. N_2 fixation was measured in 24 hour long incubations with $^{15}N-N_2$ isotope. Shown are means and standard deviations. Mean responses were compared using a one-way ANOVA. No significant difference ($\alpha = 0.05$) between treatments means is indicated by the same letter. In B3, there was no significant difference between all treatments.

Discussion

Lateral transport of DOP from the productive upwelling region to the open ocean is thought to fuel primary productivity and N₂ fixation in the oligotrophic North Atlantic (Mather et al., 2008; Reynolds et al., 2014). In this study, we investigated the availability of different DOP sources to the phytoplankton community and examined the effect of DIP and DOP addition on N₂ fixation through nutrient addition experiments.

In all six bioassays, the addition of AMP (DON+DOP treatment) considerably increased Chl *a* concentrations in comparison to the control. As this increase was not observed in treatments with DIP or DOP, we presume that the N containing part of the AMP molecule was the driver for the observed Chl *a* response. In general, nucleotides (including AMP) are known to be highly bioavailable for phytoplankton in oligotrophic regions (Björkman and Karl, 1994; 2003). The hydrolysis of AMP by phytoplankton, while also providing P, is thought to mainly supply N from the purine base to the organism (Karl and Björkman, 2002). The observed Chl *a* increase in our experiments exclusively under AMP addition confirms this assumption. Our data further show that the bulk phytoplankton community from the ETNA is limited by the availability of N, which is also suggested by the presence of excess P at five of six experimental stations. Thus, results obtained from this study add to the growing body of literature indicating that N is the proximate limiting nutrient for phytoplankton growth in the subtropical and tropical North Atlantic (e.g. Davey et al., 2008; Meyer et al., 2016; Moore et al., 2013). In addition we show that this limitation is not only alleviated by inorganic N compounds such as nitrate or ammonium, but also by the presence of DON. Interestingly, surface DON values at the sampling stations were high (~4–7 µmol L⁻¹) and markedly exceeded DON concentrations added during the nutrient treatments. Low Chl *a* concentrations (<0.5 µmol L⁻¹) at the start of the experiments indicate that this initial DON pool contained only semi-labile to refractory DON compounds, that were not readily available for phytoplankton. This observation agrees with previous studies reporting high DON concentrations in the ETNA, which appeared to be largely inaccessible for the photoautotrophic community (Letscher et al., 2013; Torres-Valdés et al., 2009). The presence of excess P is classically assumed to provide a niche for diazotrophic organisms (Codispoti, 1989; Deutsch et al., 2007). Although the oligotrophic North Atlantic is widely characterized by a deficiency of P over N, upwelling over the northwest African shelf transports waters with a low N:P

signature (originating from benthic N loss) to the surface (Jaeschke et al., 2010; Schafstall et al., 2010). In this study, different concentrations of P^* were detected in the surface of the sampling area. In the northeast, P^* was high whereas no excess phosphate or very low concentrations were detected in the south, suggesting a transition from upwelling influenced water masses closer to the shelf to oligotrophic conditions further off-shore. This is also supported by the contrasting temperature regimes between stations B1 and B5/B6. N_2 fixation was detected at all stations, but was lowest at station B2, where high initial concentrations of dissolved inorganic nutrients probably favored the growth of non-diazotrophic phytoplankton. Apart from station B2, the addition of DIP increased N_2 fixation rates in all bioassays, confirming previous results of P limitation or co-limitation in diazotrophs from the tropical Atlantic (Sañudo-Wilhelmy et al., 2001) and ETNA (Mills et al., 2004).

In almost all experiments, similar N_2 fixation responses were observed in treatments amended with DIP and DOP. Especially in bioassays conducted at stations with no/low initial P^* concentrations, N_2 fixation rates increased in equal proportions after DIP and DOP addition. This was surprising, as DIP is thought to be the preferred (i.e. more bioavailable) substrate for photoautotrophs and can be directly assimilated by the cell (Björkman and Karl, 2003). However, this seems to be different under P stress, when hydrolytic enzymes are already up-regulated. In that case, no difference between the availability of DOP and DIP seems to exist – at least not in the short time frame of our experiments.

In amendments with DON+DOP, different N_2 fixation responses were observed between experiments. In B1, B2 and B4, N_2 fixation rates did not change or decreased compared to the control. Here, the DON-fraction within the AMP molecule seemed to drive the observed response, either by favoring growth of non-diazotrophic photoautotrophs (Tyrrell, 1999) or by suppressing N_2 fixation (Dekaezemacker and Bonnet, 2011; Holl and Montoya, 2005). However, in experiments B3, B5 and B6, DON+DOP addition increased N_2 fixation rates. This suggests that the DOP-fraction within AMP exerted a greater control on N_2 fixation in experiments where there was stronger P limitation of the diazotrophic community. In experiments B5 and B6, where P limitation was strongest, no difference was observed in N_2 fixation responses between DON+DOP and the other treatments. P esters, such as G-6-P, were previously shown to be less bioavailable to the phytoplankton community than nucleotides (Björkman and Karl, 1994). However, our data indicate that

diazotrophs in the ETNA were able to equally utilize both DOP compounds, especially under stronger P limitation. This suggests that under changing environmental conditions (e.g. varying degrees of P limitation), DOP compounds are exploited differently.

Conclusion

In this study differences in the bioavailability of selected phosphorus compounds to photoautotrophs were investigated in nutrient addition bioassay experiments. We found that the bulk phytoplankton community in the ETNA is primarily limited by the availability of N, while diazotrophs are limited by P availability, confirming previous results obtained in the area. N and P limitations were not only alleviated by inorganic nutrient addition but also by amendments with dissolved organic compounds. Specifically for phosphorus we observed no difference between the bioavailability of DIP and DOP substrates used in this study (G-6-P and AMP), most notably in areas where diazotrophs were more P limited and hydrolyzing enzymes seemed to be up-regulated.

Our findings highlight the importance of implementing diazotrophic DOP utilization into model simulations predicting N_2 fixation in the global ocean (Landolfi et al., 2015; Somes and Oschlies, 2015). A more accurate estimate of the ecological niche occupied by diazotrophs will reduce uncertainties concerning input processes of fixed N into the ocean, thus facilitating a more realistic assessment of the global fixed N budget.

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Synthesis and future perspectives

In light of expanding oxygen minimum zones and the concomitant alteration of nutrient inventories in water masses influenced by oxygen deficiency, the presented doctoral dissertation was designed to investigate the influence of variable nutrient stoichiometries on primary producers and organic matter composition. The overall goal was to assess the potential of phytoplankton to modify nutrient supply anomalies and their role in coupling or decoupling sources and sinks of fixed N through modification of the P inventory. The results obtained in studies presented in Chapters I-III will be synthesized and discussed in the following sections. Subsequently, future research perspectives will be addressed.

Controls on cellular nutrient stoichiometry

Based on theoretical considerations by Klausmeier (2004), Mills and Arrigo (2010) suggested that non-Redfield production might diminish the excess of phosphate in upwelling regions. Results obtained in Chapters I and II suggest that this view is too simplistic in a dynamic ecosystem, where mechanisms other than species-specific phytoplankton nutrient uptake ratios impact nutrient cycling in the surface ocean.

In mesocosm experiments presented in Chapter I, nutrient utilization and incorporation into biomass was strongly dependent on the growth phase of primary producers. Previous studies suggested that during exponential growth resources are selectively allocated to P rich biomolecules within the assembly machinery in phytoplankton resulting in low cellular N:P ratios (Klausmeier et al., 2004; Sterner and Elser, 2002). In contrast to these studies, community PON:POP ratios were relatively constant during exponential growth and close to Redfield proportions over a large range of N:P supply ratios in our mesocosm experiments. These findings are in agreement with culture experiments (Goldman et al., 1979) and previous mesocosm studies from the ETNA and ETSP (Franz et al., 2012a) and suggest that phenotypical differences in nutrient assimilation and incorporation exist in blooming phytoplankton species such as diatoms. This observation can further be supported by theoretical work of Loladze and Elser (2011), who argue that fast growing primary producers do in fact allocate a lot of resources to the production of rRNA. Since rRNA itself is produced by protein biosynthesis, N

rich proteins need to be present simultaneously. Thus, the authors suggest that cellular stoichiometries of 16:1 can also be found in fast growing phytoplankton under nutrient saturation (Loladze and Elser, 2011). However, it has to be kept in mind that optimal growth conditions are not the norm in the marine environment and nutrient limitation is a widespread phenomenon in the surface ocean (Moore et al., 2013). Accordingly, stationary phytoplankton growth after nitrate or phosphate depletion was connected to more variable cellular stoichiometries that correlated to nutrient supply ratios in our mesocosm experiment. However, cellular stoichiometries were again not as variable as previously reported (Franz et al., 2012a; Geider and La Roche, 2002) and predominantly exceeded Redfield proportions even at very low N:P ratios.

The fact that a local balance between phenotypes, species composition and remineralization processes may average cellular deviations from Redfield proportions within the plankton ecosystem was highlighted during in situ measurements off Peru (Chapter II). At the time of the study very low inorganic nutrient ratios were encountered and upwelled water masses featured high P^* concentrations. As previously reported for the Peruvian upwelling system, a distinct succession of different phytoplankton groups was observed on transects extending perpendicular from the coast to ~150 km offshore. Contrary to previous observations determining a correlation between phytoplankton functional types and particulate organic matter stoichiometry on a transect off Peru (Franz et al., 2012b), the PON:POP ratios in surface waters were remarkably constant and close to Redfield proportions. Compared to Franz et al. (2012b), nutrient availability was higher during the study presented here, resulting in much larger phytoplankton standing stocks. When taking observations from Chapter I into account, this might suggest that the phytoplankton community (specifically close to shore) encountered nutrient replete conditions, thus exhibiting less variable particulate organic matter stoichiometries. Moreover, it has to be taken into consideration that POM can contain numerous phytoplankton and microzooplankton species and detrital particles of mixed origin. It is generally assumed that zooplankton vary much less in their elemental stoichiometry than phytoplankton (Touratier et al., 2001) and that remineralization of detritus can also reduce the stoichiometric diversity in particulate organic matter (Frigstad et al., 2011). Thus, part of the PON:POP variability that can be observed on the single species level is averaged out locally within the ecosystem and is not reflected in particulate

organic matter ratios (Fig. 1). This mechanism of ‘ecosystem averaging’ has previously been proposed as an explanation for the strong uniformity of particulate organic matter ratios (PON:POP) in seawater, despite a wide range of cellular stoichiometries in individual plankton species (Weber and Deutsch, 2010). Based on the results obtained during this dissertation, I propose that cellular stoichiometries of phytoplankton species strongly depend on nutrient supply, on the growth phase and on intraspecies variations. In the marine environment, those differences in phytoplankton N:P can be averaged over small spatial scales.

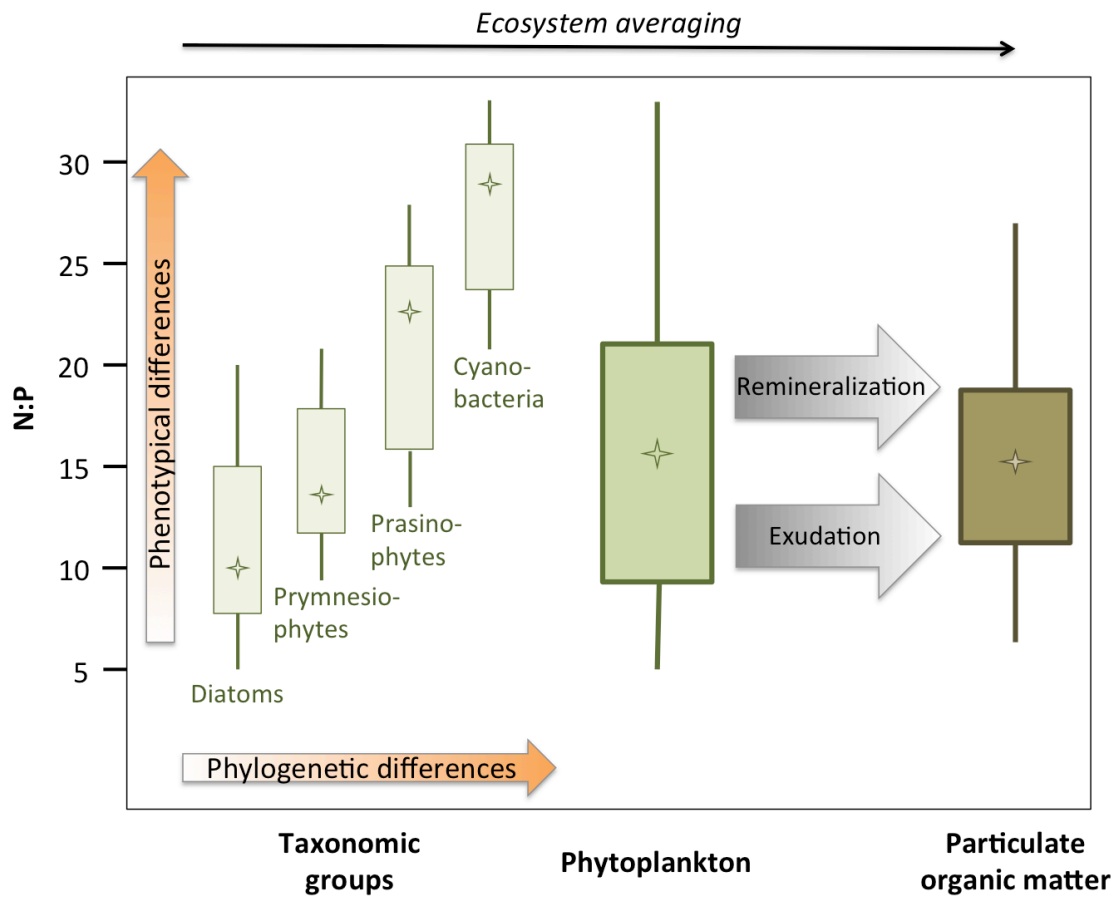


Figure 1: Conceptual figure depicting the mechanism of ecosystem averaging. The N:P variance that exists within phytoplankton species and between taxonomic groups is reduced in the overall phytoplankton assemblage. The N:P variance is further reduced in organic particles, that also comprise microzooplankton species and detrital particles, both of which vary much less in their elemental stoichiometry. Box plots indicate the 25th and 75th percentiles (box), and total range (whiskers). The stars indicate the mean.

The influence of P* on DOP dynamics

The conservation of PON:POP ratios close to Redfield proportions through ‘ecosystem averaging’ did not explain for the observed decrease of P* in the mesocosm experiments and along advection pathways in water masses off Peru. This suggested that either N was added to the systems via N₂ fixation or P was preferentially removed from the inorganic nutrient pools. Observations in Chapter I and II clearly demonstrate that a large proportion of P* can be channeled through the phytoplankton biomass into the DOP pool. A strong correlation between initial PO₄³⁻ concentrations and DOP production was observed during mesocosm experiments in the ETNA. Moreover, measurements off Peru suggested that net DOP production is enhanced in the shelf region, where P* values were highest. Luxury uptake of PO₄³⁻ by phytoplankton is commonly observed under P replete conditions (Davis et al., 2014; Franz et al., 2012a). In upwelling regions, the transfer of PO₄³⁻ to the dissolved organic pool may allow P to remain in surface waters instead of being exported with particulate organic matter (Mackey et al., 2012). DOP generated in shelf regions of upwelling systems is generally suggested to be transported laterally to the open ocean (Reynolds et al., 2014). However, observations within the Peruvian upwelling system in Chapter II indicate that a large fraction of DOP is already utilized close to the shelf because DOP concentrations decrease as water masses are transported offshore. Although little is known about the composition and bioavailability of DOP, diazotrophs were shown to scavenge P from certain organic sources (Dyhrman et al., 2006), especially when PO₄³⁻ concentrations are low. Hence, it has been suggested that relatively high DOP availability in upwelling regions creates a niche for diazotrophs, even if non-Redfield nutrient uptake reduces P* (Franz et al., 2012a). Results presented in Chapter I and III support this view and will be discussed in the next section.

N₂ fixation in the vicinity of upwelling regions

The potential spatial coupling of N gain and N loss processes in upwelling regions is a critical aspect within the framework of OMZ research. Thus, a central goal of this thesis was to assess whether water masses influenced by OMZs create a niche for diazotrophs, either by providing an excess of P over N or by supplying DOP, which is potentially bioavailable to N₂ fixers. The studies presented in Chapter I and III were designed to test these assumptions. While in mesocosm experiments (Chapter I) N and P supply was manipulated and

DOP accumulated naturally during and after the induced phytoplankton bloom, different P sources were added during bioassay experiments (Chapter III). Results obtained during both studies confirm that diazotrophs can utilize a variety of DOP compounds, including nucleotides, P esters and natural DOP compounds released by diatom dominated phytoplankton blooms. These findings also strengthen the assumption that diazotrophs potentially utilized DOP in the Peruvian upwelling region (Chapter II), which is further supported by the detection of pigments potentially belonging to diazotrophic cyanobacteria off the Peruvian coast. Together, results from all three chapters in this dissertation indicate that even if P^* concentrations are reduced through non-diazotrophic nutrient utilization (Franz et al., 2012b; Mills and Arrigo, 2010) or via the channeling of PO_4^{3-} into the dissolved organic pool in upwelling regions, diazotrophs are able to sustain N_2 fixation on organic P compounds. In principle, this confirms biogeochemical models predicting that OMZ influenced water masses provide a niche for N_2 fixers (Deutsch et al., 2001; 2007). However, it becomes apparent that the concept by Deutsch et al. (2007) needs to be extended to include not only P^* as a trigger for N_2 fixation, but also DOP. Indeed results from Chapter III imply that the N_2 fixation response to DIP and DOP supply can be on the same order of magnitude, emphasizing the significance of DOP as a nutrient source to diazotrophs. Furthermore, the results of this dissertation demonstrate that N loss and N gain processes are even more closely coupled than suggested by Deutsch et al. (2007), who argued that N_2 fixers only become competitive when bioavailable N is depleted by non-diazotrophs in the surface ocean. A closer link between N loss and N_2 fixation is supported by several observations presented in this thesis:

- 1) Diazotrophs associated to diatoms (DDAs) were determined to be the dominant N_2 fixers in the mesocosm experiment (Chapter I). N_2 fixation in these DDAs was – in contrast to the classical view – not inhibited by inorganic N compounds. This suggests that DDAs are potentially able to actively perform N_2 fixation in shelf waters of upwelling regions.
- 2) Off Peru, marker pigments of diazotrophic cyanobacteria were detected right above the shelf in the most productive area of the upwelling region and in close coupling to the diatom dominated phytoplankton community (Chapter II).
- 3) During bioassay experiments in the ETNA (Chapter III), N_2 fixation was also detected in incubations with high $NO_3^- + NO_2^-$ concentrations

($\sim 4 \mu\text{mol L}^{-1}$) and was not exclusively suppressed by the addition of DON compounds.

Other observations from upwelling regions also suggest that diazotrophs are competitive under nutrient replete conditions and are able to perform N_2 fixation when reactive N compounds are present. Increased N_2 fixation rates were determined during the upwelling periods in the eastern equatorial Atlantic (Subramaniam et al., 2013), in the Benguela- and Chilean Upwelling Systems (Moutin et al., 2008; Sohm et al., 2011) and after deep water addition during a mesocosm experiment off Gran Canaria (A. Singh, personal communication). In all cases, the excess of P over N was determined as the driver of increased N_2 fixation rates. In addition, the detection of previously unknown *Trichodesmium* phylotypes in the ETSP (Turk-Kubo et al., 2014) and evidence of heterotrophic N_2 fixation in subsurface waters of OMZs (Bonnet et al., 2013; Dekaezemacker et al., 2013; Loescher et al., 2014) add to the growing body of evidence that denitrification/anammox and N_2 fixation are closely coupled in upwelling regions. These findings may also help to fill the proposed disparity between N input and the N loss processes (Codispoti, 2007) and the gap between global N_2 fixation estimates derived from distribution of geochemical tracers and those from actual rate measurements (Mahaffey et al., 2005).

According to recent modeling studies, the tight spatial coupling of N loss and N_2 fixation, which is implied by results presented in this thesis, suggests to lead to a runaway feedback in the marine N cycle (Canfield, 2006; Landolfi et al., 2013). The authors argue that increased N_2 fixation due to excess P availability would increase organic matter export, oxygen consumption and subsequently denitrification in subsurface waters. As N_2 fixation only produces 16 moles of N per mole of P, while 120 moles of N are remineralized per mole of P during denitrification, a net loss of N would be the consequence. Upwelling of this N deficit further stimulates N_2 fixation, resulting in a positive feedback cycle that eventually lead to a declining N inventory (Canfield, 2006; Landolfi et al., 2013). The triggering of this feedback can be bypassed, however, if organic matter production and remineralization are separated (Landolfi et al., 2013). For example, the aerobic respiration of organic matter (Su et al., 2015) or the release of DON by diazotrophs and the subsequent offshore transport of DON could uncouple organic matter production and denitrification. Whether DON release by diazotrophs is an

important mechanism preventing a runaway feedback in the marine N cycle in upwelling regions remains to be resolved.

Biogeochemical and ecological consequences of expanding OMZs

In many oceanic regions, N and P availability have been shown to co-limit primary productivity (reviewed by Elser et al. (2007) and Moore et al. (2013)). In upwelling regions, however, nutrient addition experiments determined N to be the only limiting nutrient, as the sole supply of N increased primary production, while the amendment with P or N+P did not additionally stimulate biomass accumulation (Chapter I and III; Franz et al., 2012a; Thomas et al., 1974). Thus, ongoing deoxygenation and the predicted future increase in N loss processes (Capone and Hutchins, 2013; Kalvelage et al., 2011) might result in a significant decline in total primary production in upwelling regions as a consequence of reduced N supply. In addition to potential phytoplankton biomass reduction under decreased N supply, the size structure of the phytoplankton community might change. Phytoplankton cell size is a functional trait that determines the nutrient diffusion per unit of cell volume (Raven, 1998), which means that smaller cells can take up nutrients much more efficiently due to their higher surface to volume ratio (Lewis, 1976). Nutrient concentration and supply often control phytoplankton cell size and low nutrient input into the upper ocean layer often selects for phytoplankton communities dominated by picoplankton (Marañón, 2015). Moreover, Cermeño et al. (2006) showed that phytoplankton size structure shifted significantly between upwelling and downwelling seasons, with pico- and nanoplankton dominating the less productive period. In upwelling regions, large phytoplankton cells tend to dominate the system, which are grazed by large zooplankton, resulting in short food chains and an efficient energy transfer to larger consumers (Marañón, 2015; Ryther, 1969). A change towards smaller phytoplankton species in upwelling regions would have a cascading negative effect on the productivity and size structure of the pelagic food web (Canales and Law, 2015; Finkel et al., 2009), which is sustained by high standing stocks of phytoplankton species (Dugdale, 1972).

Methodological approaches: Combining experiments and field studies

During this thesis, three different approaches were used to investigate the response of phytoplankton and organic matter composition to variable nutrient stoichiometries: field surveys, mesocosm experiments and bioassay approaches. Field surveys are helpful tools to investigate the spatial dynamics and heterogeneity of ecosystem processes. Natural abundances of major taxa, species diversity and community composition can be monitored in situ while simultaneously assessing biogeochemical and physical parameters. The structure of an ecosystem can be assessed and variability of patterns over spatial scales can be observed. Drawbacks are that these observations are usually only made at a single point in time or during a brief period and single ‘snapshots’ of a system are unlikely representative of mean conditions. Thus, temporal patterns and seasonal or interannual fluctuations can often not be characterized. It can be challenging to identify mechanisms driving observed changes in biomass or organic matter composition within the natural system, because of the interplay of biotic and abiotic factors. For a better mechanistic process understanding, mesocosm experiments are a useful tool, as they provide a balance between control and realism. Under close to natural conditions, responses of lower trophic levels (bacteria, phytoplankton, zooplankton) to certain environmental factors can be studied over longer time scales of several days to weeks or months. Mesocosm experiments are useful manipulation facilities because they are more representative of the natural environment than highly controlled, small-scale laboratory experiments and better assessable than in situ field surveys. However, mesocosm experiments also have a number of drawbacks. The small size of the experimental facilities creates different mixing regimes and can lead to artificial sinking of particles (Watts and Bigg, 2001) and wall growth can substantially decrease nutrient availability for planktonic organisms in mesocosms (Chen et al., 1997). Ecosystem successions during mesocosm experiments may not be fully resolved because of the short experimental duration or may not actually represent temporal dynamics under natural conditions and could only be artifacts of the controlled environment (Duarte et al., 1997). Thus, the extrapolation from these artificial systems to complex natural systems needs to be made with significant considerations.

Small-scale experiments such as bioassays are commonly used in marine ecology and biogeochemistry to determine the effect of one factor (e.g. light,

CO₂ concentration, nutrient availability) on a species or small community. They are relatively easy to set up and carry out, thus enabling a wide variety of processes to be investigated under very controlled conditions. As bioassays are often conducted on a small scale (mL to L) and under highly artificial growth conditions, it has been argued that the observed responses can have little to do with the overall ecology of the system in which the experiments are conducted (Carpenter, 1996). Thus, generalizations from these experiments must be drawn with caution.

When all these methodological approaches are combined and experimental findings are supported by observational studies, a comprehensive picture of ecosystem processes and dynamics can be gained. This was nicely illustrated in this dissertation: in situ observations off Peru showed that P* was reduced in water masses further offshore, whereas DOP seemed to accumulate close to the shelf. During the mesocosm experiments it was possible to demonstrate that P* concentration and DOP production are positively correlated as phytoplankton take up P* and release it as DOP, confirming one of the proposed mechanisms of P* consumption. At the same time we found indications that diazotrophs were able to utilize DOP in the mesocosms. The availability of selected DOP compounds to N₂ fixers was then confirmed during the bioassay experiments. Hence, combining small-scale experiments, field studies and larger-scale in situ observations while interpreting data within the context of knowledge of the overall system can facilitate a better understanding of the complexity of marine ecosystems.

Future research perspectives

While results obtained in the course of this dissertation strongly suggest that DOP supply supports N₂ fixation in the ETNA, only indirect evidence supports the assumption that P* and/or DOP consumption are coupled to diazotrophy in the ETSP. Moreover, little is known about the magnitude of N₂ fixation in this area and the proportion of auto- and heterotrophic N₂ fixation to the overall N₂ fixation rate. DOM bioavailability, supply and consumption by diazotrophs may play a critical role in organic matter turnover and nutrient cycling and should be given attention in future studies. The suggested approaches are described more in detail in the following.

Characterization of the dissolved organic matter pool

The bioavailability of dissolved organic nitrogen and phosphorus to phytoplankton and nitrogen fixers (Chapter III) sheds new light on our understanding of marine primary production and highlight the importance of considering both organic and inorganic nutrients as factors regulating phytoplankton productivity and nutrient cycling. While inorganic nutrient concentrations are commonly determined in the majority of oceanographic surveys, dissolved organic nutrients are often disregarded and the systematic mapping of DON and DOP distribution in the ocean basins has only been done in a few studies (Moutin et al., 2008; Torres-Valdés et al., 2009; Vidal et al., 1999). Thus, little is known about the global significance and bioavailability of dissolved organic nitrogen and phosphorus compounds. For a comprehensive understanding of nutrient cycling and its effect on primary productivity, the determination of these components should be routinely included in future studies. Specifically in upwelling regions, little is known about the composition and bioavailability of different organic matter compounds. The chemical characterization of DOM could be assessed through Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), while the labile fractions of DON and DOP could be determined through amino acid and P monoester measurements.

The role of diazotrophs in DON and DOP cycling

The release of DON by diazotrophs and the subsequent offshore transport of diazotroph-derived DON could uncouple organic matter production and denitrification. Hence, the trigger of the runaway feedback in the marine N cycle (Canfield, 2006; Landolfi et al., 2013) may be bypassed. While release of newly fixed N as amino acids and dissolved inorganic nitrogen has been frequently observed in diazotrophic cyanobacteria (Benavides et al., 2013; Glibert and Bronk, 1994; Konno et al., 2010), the magnitude of this release is highly variable and not well determined (10-80%). Thus, investigating the fate of diazotrophic derived fixed N in upwelling regions should be a major focus of future research, also with respect to the concomitant underestimation of N₂ fixation rates through DON exudation (Benavides et al., 2013). Following the work of a recently conducted mesocosm experiment, this could be done in ¹⁵N₂-labelled “diazotrophic-derived nitrogen transfer experiments” in combination with the amino acid measurements and high-resolution nanometer scale secondary ion mass spectrometry (nanoSIMS) (Berthelot et al., 2016; Bonnet et

al., 2015). With the help of enzyme-labeled fluorescence (ELF) incubations, the activity of DOP hydrolyzing enzymes (e.g. alkaline phosphatase) can be determined at the single cell level (Girault et al., 2012). Complemented with diazotroph-derived N transfer experiments, this approach could provide valuable information on both the role of DOP as nutrient source for diazotrophs and the role of newly fixed N in the ETSP. Combining the proposed experiments with large-scale N₂ fixation rate measurements and assessments of DON and DOP concentrations during field surveys would help to get a better understanding of the importance of DOP and DON pools to microbial processes in the ETSP.

Impacts on export production

Identifying the consequences of changing N supply through ocean deoxygenation is not only crucial for our understanding of possible changes in ocean ecosystems, but is also necessary for a more accurate flux estimations in the marine carbon cycle (Lachkar and Gruber, 2012). Biomass production and cell size are two key factors that modulate export production in plankton communities and are both closely linked to nutrient concentration and supply. Hence, changing N supply may influence the export efficiency of particulate organic matter and the magnitude of carbon transfer from the surface to the ocean interior (Muller Karger et al., 2005). Future research should therefore not only focus on community composition and stoichiometric changes, but should also take into account the role that phytoplankton and particle size distribution dynamics play within plankton communities. During experiments, field- or long term monitoring studies, the size distribution of phytoplankton can be determined with little effort either by size fractionated filtering of Chl *a* samples or via flow cytometry, while the size distribution of larger particles and zooplankton can be determined with automated image techniques like the Underwater Vision Profilers (UVP) and ZooScan.

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Eidesstattliche Erklärung

Hiermit bestätige ich, dass die vorliegende Arbeit mit dem Titel:

**Changes in nutrient stoichiometry:
phytoplankton & organic matter dynamics
in coastal upwelling systems**

von mir selbstständig verfasst worden ist und keine weiteren Quellen und Hilfsmittel als die angegebenen verwendet wurden. Ferner versichere ich, dass die Arbeit unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden ist und weder im Rahmen eines Prüfungsverfahrens an anderer Stelle vorgelegt noch veröffentlicht wurde. Veröffentlichte oder zur Veröffentlichung eingereichte Manuskripte wurden kenntlich gemacht.

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